

PROTON TRANSFER AND OPERATIONAL PH
IN AQUEOUS-ORGANIC SOLVENT SYSTEMS

By

ROBERT W. TOWNSEND

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For my parents Leland and Frances Townsend, who dared me to dream, and my daughter, Harmony, for the desire. This is for you.

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Robert W. Townsend

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The lowest excited singlet state properties of 3 isomeric hydroxybiphenyls and 6-methoxyquinoline were examined in pure water and mixed aqueous-organic cosolvent systems with a combination of steady-state and time-resolved fluorescence techniques. The results indicate that each of these probes displays proton transfer in the lowest excited singlet state in both the aqueous and mixed aqueous-organic media. One finds that the pseudo-first-order acid dissociation (or base hydrolysis) reaction is strongly solvent dependent, yet independent of the acidity (or basicity) of the solution. As the mole fraction of organic cosolvent is increased, the rate constant decreases biexponentially, eventually going to zero. The slope of the logarithm of the dissociation (or hydrolysis) rate constant against the mole fraction of organic cosolvent was found to

be proportional to the ratio of the diffusion volume of the excited probe to the molecular volume of the organic cosolvent. The logarithm of the dissociation (or hydrolysis) rate constant against the logarithm of the water activity can be used to characterize water structure and determine the number of water molecules that participate in the reaction at any given solvent composition. The second order reprotonation reaction, on the other hand, depends predominantly on solution acidity. In addition, one also finds that the back reaction depends upon the continuum properties of the solvent. Fortunately, steady-state and time-resolved fluorescence methodologies allow reprotonation to be treated independent of dissociation. The linear relationship between the ratio of the relative fluorescence efficiencies of the acid and conjugate base and the hydrogen ion concentration is obtained if proper Brønsted activity factors are included in the relationship. These factors can be calculated from classical electrostatics and are simple powers of the activity coefficients necessary to convert the formal hydrogen ion concentration to hydrogen ion activity. Using this approach, it is possible to calculate pH from hydrogen ion concentration in aqueous organic solutions.

CHAPTER 1 INTRODUCTION

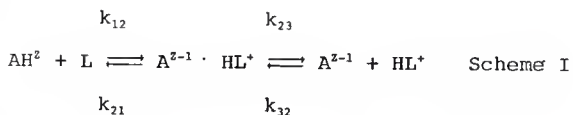
Mixed aqueous-organic solvent systems are widely used in the pharmaceutical sciences. They alter chemical properties (stability and solubility) and therefore are frequently used as vehicles to affect stabilization, dissolution, dispersion and delivery of drugs. In addition, mixed solvent systems are utilized as reaction media for synthesis and as extraction media or mobile phase to aid in the isolation and separation of complex mixtures of compounds. However, much of the current knowledge regarding mixed solvent systems is still subject to speculation.

The kinetics of excited state proton transfer are affected by solvent composition in ways which reflect the solvations of the reactants and products and the influences of their structures on ionic or molecular diffusion.

If these relationships can be evaluated accurately, a greater understanding of the nature of solute-solvent and solvent-solvent relationships as well as a better predictability of properties of solutes (drugs) in aqueous, mixed aqueous-organic and more complex biological systems may result.

In the past, excited state proton transfer has been utilized as a simple means of probing solvation and solvent structure, in aqueous and mixed aqueous-organic solvent systems. For example, in 1963, using a steady-state kinetic approach to excited state proton transfer, Urban and Weller (1) examined the behavior of acidic probes in mixed solvents. In their investigation of proton transfer from ammonium pyrene derivatives in methanol, ethanol, n-propanol and n-butanol, they found that the dissociation rate constants decreased concurrently with temperature and that this decrease was dependent upon the chemical nature of the solvent.

The proton transfer process was depicted as follows in scheme I:



where the acid (AH) with a valence (Z) donates a proton to the accepting solvent (L). Resolution of the probe was considered to occur concurrently with the proton transfer process because of a change in the charge of the reacting species. It was also felt that the reduction in the dissociation rate constant with temperature was due to a retardation in the resolution process based upon the observed similarities that were found between the measured

activation energies for proton transfer and dielectric relaxation in each of the solvents investigated.

In 1965, Trieff and Sundheim (2) determined the excited state proton transfer rate and equilibrium constants for the weakly acidic 2-naphthol (2N) in increasing amounts of organic cosolvent using the steady-state technique. The rate of deprotonation decreased with increasing amounts of organic cosolvent yet no change was found in the reprotonation process. The latter observation contradicted the theoretical predictions of Weller (3) and Williamson and La Mer (4) for diffusion controlled reactions.

The observed results were rationalized in terms of a model which involved a preliminary ionization step, followed by reaction of the ion with water. It was concluded that the bulk dielectric constant must play a role in the ionization process of 2N because in the solvents investigated, dioxane, on a molar basis, exhibited a greater inhibitory effect on the deprotonation process than did glycerol or methanol.

In 1978, Huppert et al. (5), with the help of a picosecond spectroscopic technique, time-resolved emission spectroscopy (TRES) and complimentary steady-state fluorescence spectroscopy, measured the rate of proton dissociation from 8-hydroxy-1,3,6-pyrene trisulfonate (HPTS) in mixed aqueous ethanol solutions. They found from semilogarithmic plots of the rates of proton dissociation as

a function of the mole fraction of ethanol that proton dissociation decreases sharply as the ethanolic mole fraction is increased and speculated that the rate of proton transfer was directly influenced by the ability of water to form clusters. They cited the prototropic conductivity experiments by Erdey-Gruz and Lengyel (6) which give support to a cluster order in water and referred to the theoretical quantum mechanical calculations of Kaemer and Diereksen (7), along with the ion clustering experiment conducted by Kebarle (8), for the existence of water dimers as an energetically favored species of water relative to the monomer species.

In 1981, Huppert et al. (9) extended studies in mixed solvent systems by examining the excited state proton transfer of 2N, 2-naphthol-6-sulfonate (2N6S) and HPTS in aqueous alcohol systems. Semilogarithmic plots of the dissociation rate constant versus the alcoholic mole fraction were found to be linear with slopes, independent of the probe, increasing in the following order: methanol, ethanol and n-propanol. Since the probes in this study (i.e., weak acids with $pK' > 0$) did not exhibit excited state behavior in the pure organic solvents and since the bulk dielectric constant could not account for the reduction in proton transfer rate, they felt that the dependence of proton transfer in mixed solvents was contingent solely upon

the properties of water and reasoned that a structural model of water in which diffusion of protons is mediated through rapid exchange of hydrogen bonds-covalent bonds throughout the quasistatic continuous network of hydrogen bonds must be responsible for proton transport. Support for a structural model came from Stillinger (10) who had concurred that the distinct structural features and properties of liquid water are related to the shape of the individual water molecules and their tendency to hydrogen bond with each other. Further, the x-ray and neutron diffraction studies on water conducted by Narten and Levy (11), the subpicosecond experiments on the photoejection of electrons in aqueous solutions by Wiesenfeld and Ippen (12) and the theoretical studies conducted by Lie and Clementi (13) as well as those from Stillinger and Rehman (14) provided strong evidence in support of a local tetrahedral ordering of water, primarily through hydrogen bonds. In addition, the dynamic flow studies of Searcy and Fenn (15) demonstrated the existence of $H^+(H_2O)_n$ clusters which vary in size from one to more than twenty water molecules. Finally, the quantum mechanical calculations of Newton and Ehrenson (16) in addition to those of Kraemer and Diereksen (7) along with the results of Kebarle (17) provided thermochemical evidence in support of the proton hydration process.

With this background, Huppert and coworkers considered

that in the final state of proton transfer, the excited anionic species and a proton would exist hydrated in a complex of n water molecules. Further, since the free energy of the reaction is dependent on the enthalpy of proton hydration and since a linear relationship between the dissociation rate constant and the mole fraction of organic cosolvent was observed, they concluded that the addition of organic solvents to the water network must disrupt the hydrogen bonding network of water, leading to a decrease in water cluster size, and that such behavior directly affects the rate of proton transfer, possibly by delaying the rate of proton hydration.

In 1981, Schulman and Vogt (18) investigated the proton transfer kinetics of 2- and 4-quinolone in moderately concentrated acid solutions using steady-state fluorimetry. By assuming that the rate of dissociation was limited by the rate at which the dissociating acid was hydrated to form the transition state (or the solvent-separated ion-pair which is the encounter complex of the fully hydrated proton and the fully hydrated conjugate base), they found that they could quantitate excited state proton transfer. This model was later extended to the excited state investigation of molecules containing either neutral or monocationic conjugate bases and monocationic or dicationic conjugate acids, respectively (19,20). The approach employed a

relationship between the relative quantum yield ratios of the conjugate acid and base with the Hammett acidity function h_0 and the hydration requirements for the reaction of interest and the dissociation of the Hammett indicator. They found that the hydration requirements in concentrated acid solutions for proton transfer ranged from 1 to 8 water molecules. To explain this observation, it was suggested that one or more of the reactants may not be in its maximum state of hydration because free water in concentrated acids is scarce.

In 1984, Huppert et al. (21) investigated the rate of proton transfer of 2N, 2N6S, 2-naphthol-3,6-disulfonate (2N36DS), 2-naphthol-6,8-disulfonate (2N68DS) and HPTS in concentrated electrolyte solutions at $\text{pH} \gg \text{pK}'$, where pK' is the pK of the probe in the excited state. In this study, they found that the dissociation rate constant was linearly related to the chemical potential of water and the following relationship was found to hold

$$\log(k) = \log(k_0) - r \log(a_w) \quad (1.1)$$

where k and k_0 are the rate constants for excited state deprotonation in the salt solutions and in pure water, respectively. The slope r is an empirically determined constant, which varied from 6-10 depending on the probe, and a_w is the activity of water. It was observed that probes

containing hydrophilic moieties in proximity to the dissociating proton yielded smaller values for r . Thus, r was deemed the probability of proton transfer from the excited probe to nearby water molecules recognized as being present at different degrees of aggregation and ordered at a proper distance from the dissociating proton. Further, it was reasoned that if proton transfer was considered to occur inside a coulombic cage, then the decrease in proton transfer with increasing salt concentration must be related to ion-pair formation (a process in which the O-H bond of the excited probe forms a hydrogen bond with the nearest molecule which itself is hydrogen bonded to nearby water molecules). However, according to Eigen (22) a combination of electrostatic interactions and reduced proton diffusibility, effects which dominate ion-pair recombination and ion-pair separation, would accelerate the rate of proton dissociation. Thus, to support their ion-pair concept, they cited Conway (23), who attributed the enthalpy difference between proton hydration and hydronium ion formation to a further solvation of the hydronium ion by additional molecules of water. Finally, with the arguments developed above as well as those from their previous publications (5,9,21) and the experimental evidence presented, they claimed that the hydrated complex of a proton in dilute electrolyte solutions varies in size, but that it is

composed of no more than 10 molecules of water.

In biological investigations, Huppert and coworkers (24,25) attempted to characterize the properties of water within poorly hydrated binding sites by making use of the linear relationship that was found between the dissociation rate constant and the activity of water in electrolyte solutions (equation (1.1)). From these studies they reported that the ability of water to hydrate dissociating protons within the binding sites of bovine serum albumin and apomyoglobin was equivalent to the ability of water to hydrate protons in salt solutions where water had activities of 0.8 and 0.68, respectively.

In 1984, Bardez et al. (26) tried to extend this relationship to reverse micelles using phase resolved and steady-state fluorimetry. Preliminary studies on the proton transfer of pyranine suggested that the activity of solubilized water in reverse liposomes was in good agreement with both the activities yielded by measurement of vapor pressures (27) and those calculated from the theoretical electrostatic model of Jönsson and Wennerström (28). However, in a subsequent investigation with 2N and its sulfonated derivatives (2N6S and 2N68S), Bardez et al. (29) found that both the efficiency and kinetics of proton transfer were strongly dependent upon the localization of the probe within the micelle. It was concluded that the

validity of Huppert's method for determining the activity of water in reversed micelles should be used with some degree of caution.

Recently, Politi and Chaimovich (30) examined the rate of proton dissociation in aqueous urea solutions with HPTS and found that the forward rate constant could not be related unequivocally with the activity of water. Instead, a urea-water cluster with properties similar to pure water was proposed to account for the observed behavior. It was concluded, that since the effects of the groups at the interface on the properties of surrounding water are unknown, that any information obtained by proton transfer or related studies which probe the properties of an interface must be regarded with great care.

In 1984, in an ongoing attempt to more fully comprehend the proton transfer process, Huppert et al. (31) carried out picosecond studies of HPTS in both water and deuterated water at pressures up to the ice transition point. They found that proton dissociation was dependent upon a specific water structure, in support of previous investigations (5,9,21) and proposed the following model. The first step in proton transfer was considered to be the transfer of a proton from an excited probe to an adjacent properly aligned water molecule forming H_3O^+ . Such behavior would affect the local solvent environment, which would then lead to further

structural changes and increases in the proton hydration cluster size. They then suggested that successive proton transfers between large labile hydration complexes would increase the distance (r) between the excited anion and the proton to the point where the Coulomb field interaction or R_0 (the "effective range" of the electrostatic interaction potential between the excited anion and the proton) is smaller than the thermal energy, and that under these conditions, the proton will escape. They concluded that the rate-determining step in proton transfer for the proposed model must be the rate at which the proton is transferred to the adjacent properly aligned water molecule, and that this step ultimately depends upon the donor-acceptor distance.

In 1985, Robinson and coworkers (32) examined the proton transfer of 2N in the pH independent portion of the titration curve ($\text{pH} \gg \text{pK}'$, where the dissociation process dominates at differing temperatures) in methanol-water mixtures using the picosecond spectroscopic technique, time correlated single photon counting accompanied by steady-state fluorescence spectroscopy. They observed an increase in the proton transfer rate with increasing temperature which could not be explained by the temperature dependence of pH as described by Kolthoff and Rosenblum (33) because of the initial experimental conditions. They also found that as the alcoholic component of the reaction mixture was

increased, the effect of temperature on the proton transfer rate decreased. This behavior coincided with observations made earlier by Urban and Weller (1). They also cited that the rate of proton transfer decreased as the methanol concentration increased which served to validate previously claims (5,9). From this study, Robinson and coworkers concluded that although solvent structure plays a role in the proton transfer, the water cluster model $(H_3O^+[H_2O]_n, n > 1)$ proposed by Huppert et al. (9,21) fails to provide adequate details about the cluster size or its specific relationship to proton kinetics. In an effort to provide additional information about the proton transfer process, they adapted a Markov random walk theory previously used in the kinetic analysis of electron transfer (34-36). Results indicated that a 4 ± 1 member water cluster was the primary proton acceptor. In support, they cited Eigen and coworkers (37-39), who suggested that the condition for proton migration is a disturbed structure of water represented by a hydrogen complex which is comprised of exactly four water molecules.

Finally, the observed activation energy for proton transfer in pure water (3.45 Kcal/mole) was attributed to the energy required to reorganize the hydrogen bonded water network to form the proton accepting cluster. From this they implied that a specific structure of the critical 4 ± 1

molecule water cluster apparently determines the rate of proton transfer in aqueous media. Any additional water or cosolvent molecules must exist in secondary coordination shells and therefore, they only play secondary roles in the proton transfer process.

In 1986, Shizuka et al. (40) investigated the proton dissociation reaction of 1- and 2-naphthol in the presence of NaCl at 300 K by means of nanosecond and picosecond spectroscopy with steady-state fluorimetry. The observed proton dissociation rates of 1- and 2-naphthol decreased considerably upon addition of NaCl, in agreement with Huppert et al. (21). It was found that k_1/k_1^0 , when plotted as a function of NaCl concentration, decreased linearly and that this behavior could be described by the following equation

$$k_1 = k_1^0(1 - \alpha[\text{NaCl}]) \quad (1.2)$$

where k_1 and k_1^0 represent the proton dissociation rate constants with and without NaCl and α denotes a rate parameter which is determined from the slope of the line. This equation holds for $\alpha[\text{NaCl}] \leq 1$, and α was found to be 0.2 for the naphthols.

Shizuka et al. (40) implied that proton transfer from naphthols to water clusters is a second order process.

Consequently, the rate constant in pure water is given by

$$k_1^0 = k_0 [(H_2O)_n] \quad (1.3)$$

where k_0 represents the second order rate constant in units of $M^{-1}s^{-1}$ and $[(H_2O)_n]$ denotes the concentration of water clusters. Evidence for a 4 member water cluster had been provided (32,38,39). Further, Huppert et al. (21) had suggested that protons produced in the dissociation process become entrapped in clusters of up to 10 molecules of water. They figured that it was reasonable to assume that the destruction of proton-accepting water clusters by NaCl shuts down proton transfer and that because of this, the proton transfer rate can be expressed as

$$k_1 = k_0 [(H_2O)_4] (1 - \alpha[NaCl]) \quad (1.4)$$

where $\alpha[NaCl] \leq 1$.

Shizuka et al. (40) also speculated that the concentration of water clusters could be estimated to be 13.8 M in pure water; and since $\alpha = 0.2$, they infer that in a 1.0 M NaCl solution 20% of the water clusters (2.7 M) were destroyed, which leads to the production of 11 water molecules that hydrate the Na^+ and Cl^- ions. In their investigation, they report that the average hydration numbers for the Na^+ and Cl^- ions were 4.4 and 6.6,

respectively. These values are comparable to literature values obtained through x-ray diffraction studies (41,42).

Around the same time, Pines et al. (43) reported the excited state behavior of the heterocyclic bases, acridine and 6-methoxyquinoline in both methanol-water and concentrated electrolyte solutions using steady-state and time-resolved emission spectroscopy. The rate of heterocyclic protonation was noted to decrease with increasing methanol concentrations. However, the investigators felt that the role of water on the rate of proton abstraction could not be properly evaluated because the probability of nonradiative decay $S_1 \rightarrow S_0$ or triplet formation $S_1 \rightarrow T_1$ is higher in the nonpolar organic solvents than it is in water with heterocyclic compounds (44-46). They turned instead to a series of concentrated electrolyte solutions and found that the rates of protonation for the heterocyclic bases increased with increasing salt concentrations. From this observation, it was suggested that the addition of strong electrolytes to water decreased the concentration of free bulk water as a result of the ion hydrating process. Therefore, they claimed that water molecules that hydrate cations tend to be more acidic than free bulk water because they increase the rate of proton transfer.

In 1986, Robinson and coworkers (47-50) presented a

series of papers in support of a new proton hydration model for excited state proton transfer. In this model, the direct kinetic product of the acid dissociation is considered to be H_3O_4^+ ion. Further, they argue that photokinetic experiments on ultrafast timescales suggest that the integrity of the quasi-tetrahedral oxygen structure of liquid water controls the proton hydration process. Support for this belief stems from the early works of Kamb (51) and Triolo and Narten (52) who inferred that a key to understanding the dynamic processes in water may be the stability, on characteristic timescales, of the five-membered quasitetrahedral arrangement of oxygen atoms. With the oxygen atoms forming a more or less static background, rapid reorientational motions in the liquid phase occur primarily through the motions of the hydrogen atoms. This implies that proton transfer and other properties of liquid water, such as dielectric relaxation, (53) shear viscosity (54,55) and spin-lattice relaxation processes (56,57) are dependent upon these rapid reorientational motions.

To help comprehend the temperature dependence of these dynamic processes in pure liquid water, Robinson et al. (47) developed an analytical approximation to a temperature dependent rotational rate parameter $\kappa(T)$ which has the pseudo-Arrhenius form

$$\kappa(T) = \kappa^0(T) \exp(-\Delta H_w^*/RT) \quad (1.5)$$

where κ^0 is the experimental prefactor, ΔH_w^* is the kinetic molar enthalpy of activation, R is the gas constant and T is the absolute temperature. They felt that the temperature dependent rate parameter which is related to the Debye relaxation time ($\tau_D = \kappa(T)^{-1}$) simplifies the multitude of vibrations and structures of water to a single set of temperature-sensitive frequencies while maintaining much of the anomalous physics associated with liquid water. However, since most dynamical and transport properties associated with liquid water do not exhibit typical Arrhenius behavior, they turned to the mathematical models developed for supercooled liquid water by Lang and Lüdeman (56-58) and Angell and coworkers (59-61) for support because they approximate the thermodynamic and kinetic data over a wider range of temperatures. The heat capacity data generated by Oquini and Angell (61) appear to be of central importance in support of their model.

The differences observed between the calculated heat capacities and those obtained by Oquini and Angell (61) were speculated to arise from an assumption used in the model development which fails to include contributions from five intermolecular vibrations (water exists as a simple rotor).

However, the level of agreement, which existed between the two sets of data seemed to indicate that the temperature dependence observed in the data could account for the anomalies associated with liquid water. Further, investigations on the temperature dependence of proton recombination and proton-induced quenching for 2-naphtholate (50) showed that the dielectric relaxation times τ_d listed by Hasted (53) are essentially proportional to the newly developed pseudo-Arrhenius rate parameter over the temperature range of interest. Therefore, they concluded that their simplistic model provides a basis through which the temperature dependent properties of water can be understood, and that cooperative hydrogen bonding produces the anomalous behaviors associated with water. They also indicate that this cooperativity leads to an effective hindering potential which is generated through the hindered rotational motions of hydrogen bonds and that this behavior, in itself, provides the foundations from which the kinetic and thermodynamic properties associated with water in the pure and binary states arise.

In a subsequent paper, Robinson et al. (48) derive a set of absolute rate expressions for proton transfer processes which incorporate the Debye relaxation time, τ_D , and which correlate the dissociation and recombination rate constants of weak acids with thermodynamic ionization

enthalpies (ΔH_i^0) and entropies (ΔS_i^0).

The dissociation and recombination rate constants are defined as follows:

$$k_{dis} = \Omega \tau_D^{-1} \exp(\Delta S_i^0/R) \exp(-\Delta H_{dis}^*/RT) \quad (1.6)$$

$$k_{rec} = \Omega \tau_D^{-1} \exp[-(\Delta H_{dis}^* - \Delta H_i^0)/RT] \quad (1.7)$$

where Ω is a mobility/steric factor introduced by Eigen and Kustin (62); τ_D is the Debye relaxation time (which is related to the temperature dependent rotational rate parameter $\kappa(T)$, $\tau_D = \kappa^{-1}$); ΔS_i^0 and ΔH_i^0 are the thermodynamic entropy and enthalpy values for acid ionization; and ΔH_{dis}^* is the kinetic enthalpy for acid dissociation. Standard-state conditions require unit molarities throughout.

Support for the new rate expressions was based upon a review of weak electrolytes by Harned and Owen (63) which revealed that many weak univalent acids at room temperature have enthalpies of ionization near zero leaving only entropic barriers of ≈ -20 cal/mole to stand in the way of acid dissociation. This behavior was considered to be normal, and the group turned to the picosecond investigations on 1- and 2-naphthol conducted by Webb et al. (64) along with their previous work on the hydration dynamics of protons from the photon initiated acids, 1-and

2-naphthol in water/alcohol mixtures (49), to suggest that naphthols fit the weak acid criteria.

When proton dissociation and recombination rates are described in terms of the Arrhenius equation, where $\Delta H_i^\circ \approx [\Delta H_{dis}^i - \Delta H_{rec}^i]$, Robinson and coworkers (48) show that the free energy of dissociation ($\Delta G_i^\circ \geq 0$) for the naphthols is consistent with Harned and Owen's weak acid picture. They also show that calculations from the Arrhenius equation provide additional evidence for a hydration model in which a solvent rotational bottleneck stands in the way of the overall ion hydration reaction. For example, the inverse of basic rates, i.e., rates that the dissociation process would have if it were unencumbered by an entropy or enthalpy of activation, are roughly equal to the Debye correlational times τ_D for pure H_2O and D_2O . This is consistent with results obtained by Eigen et al. (62,65), who found that recombination rates are dependent upon frictional diffusion, a process which can be described by the Debye equation.

Robinson and coworkers are quick to point out that Eigen's indirect methods for determining the dissociation rate fails to provide adequate detail about ion hydration. They infer that the mixed alcohol-water solvent systems used throughout their studies serve as effective probes in the ion hydration process because the sterically bulky alcohol molecules require more time than is available in the excited

state to disentangle and reorient to surround the small charge. Further, with their new analysis technique (34-36) they indicate that the water cluster H_3O_4^+ is the direct kinetic product of acid dissociation. Reviews by Conway (23) and Newton and Ehrenson (16) lend support to H_3O_4^+ as a specific entity for the hydrated proton. In addition, recent gas phase studies by Kebarle (66) provide firm thermochemical evidence for successive binding of water molecules $(\text{H}_2\text{O})_n$ to H_3O^+ . Further, the theoretical calculations by Pople (67-69) and Clementi (70) appear to confirm that these energy relationships exist. Robinson and coworkers (48) conclude that the the hydration model currently provides the best means through which ion solvation can be understood.

In 1989, Lee (71) conducted picosecond studies of 2N in various salt solutions at different temperatures in an attempt to elucidate the hydration structure of an ion in an electrolyte solution. Proton transfer was found to decrease with increasing salt concentrations, consistent with the results obtained earlier by Huppert et al. (21) and Shizuka et al. (40). The relationship that Huppert used to correlate the dissociation rate constant with the activity of water was redefined in terms of the osmotic coefficient h of the solutions,

$$k_{dis} = k_{dis}^0 \exp[-nh\sum m_i v_i / 55.56] \quad (1.8)$$

where k_{dis} and k_{dis}^0 are the rates of proton dissociation in water with and without salt, n is the proton hydration number, and m_i and v_i are the molality and the stoichiometric number of the ions, respectively. Plots of $\log(k_{dis})$ versus $\log(a_w)$ of 2N in NaCl at differing temperatures were found to coincide with Huppert's work, however, Lee questioned the validity of this model because n was found to be solvent dependent and because the modified osmotic equation gave dissociation rates that were greater than observed rates in LiCl, MgCl₂ and CaCl₂ solutions.

To help explain the dynamics between the proton dissociation-hydration and the ion hydration processes during the lifetime of the excited state, kinetic solvation numbers as proposed by Taube (72) were generated for each electrolyte system. Lee (71) inferred that in ion solvation only the most loosely attached water molecules can detach from the ion and reorient to hydrate the proton during the lifetime of the excited state, and that this would constitute an intermediate region which bridges the gap between the strongly bound primary solvation shell and the bulk region where the solvent properties are retained. Retardation of the dissociation rate of 2N in increasing

concentration of salt thus results from the loss of free water, i.e., water used in ion hydration.

Lee (71), in support of Pines et al. (43), suggests that cations inhibit dissociation to a greater extent than anions because they compete with protons for the same negative part of the water molecule. Experimental evidence in NaCl and NaClO₃ solutions support this notion because the only noticeable difference in the dissociation process of 2N could be attributed to ionic quenching parameters. Thus, it was concluded that kinetic solvation numbers provide a more effective means through which the various salt effects on the deprotonation process can be explained.

In 1990, Robinson and coworkers (73) extended the hydration model to a more complicated weak acid system (i.e., one in which intramolecular hydrogen bonding interferes with proton transfer). Using picosecond laser technology, they investigated the excited state behavior of 1-naphthol-2-sulfonic acid in ethanol/water solvent systems at different temperatures. When these results were compared with the results obtained with 1-naphthol (49), it was found that the intramolecular hydrogen bonding in the sulfonate derivative sterically reduced the extramolecular proton dissociation and recombination rates. However, the overall rate was still controlled by the reorientational motions of the adjacent water molecules; and, if dissociation was to

occur, a cluster of 4 ± 1 water molecules was required to solvate the transferring proton. Thus, this investigation provided additional support to their proposed hydration model for endothermically produced protons in aqueous environments.

Recently, Schulman and Kelly (74,75) investigated the excited state behavior of 2-naphthol and some sulfonated derivatives (2-naphthol-6-sulfonate, 2-naphthol-8-sulfonate, 2-naphthol-3,6-disulfonate and 2-naphthol-6,8-disulfonate) in dimethyl sulfoxide/water and ethanol/water mixtures using steady-state fluorimetry. It was found that semilogarithmic plots of the relative quantum yield ratios $(\phi'/\phi'_0)/(\phi/\phi_0)$ versus the organic mole fraction decreased linearly and that this behavior could be fit to the following empirical relationship

$$\log\left(\frac{\phi'/\phi'_0}{\phi/\phi_0}\right) = mX_{org} = \log(k\tau_0) \quad (1.9)$$

where ϕ'/ϕ'_0 and ϕ/ϕ_0 are the relative quantum yields of the conjugate base and the acid, respectively; m is the slope and X is the mole fraction of the organic solvent. Schulman and Kelly suggested that the separation of the initial ionization products by frictional diffusion was too slow to allow dissociation to compete with recombination and that

the much faster proton jump (Grotthus) mechanisms, which imply an extended hydrogen bonded network in the solvent, are required for proton transfer to occur on the nanosecond time scale. In addition, it was felt that the slope represented some energetic parameter and since equation (1.9) was similar to equation (1.1), which relates the activity of water to the dissociation rate constant in concentrated electrolyte solutions (21), it seemed reasonable to equate the two equations through the following relationship:

$$r \log(a_w) = mX_{org} \quad (1.10)$$

Initial results in an ethanol-water system suggest that equation (1.10) may be valid. For example, a plot of $\log(k\tau_0)$ vs. $\log(a_w)$ was linear over a narrow range of the ethanol mole fraction. The slope, r , which was designated as the total number of water molecules involved in the proton transfer reaction, was found to be 12.4. One would expect a much smaller value of r (3 or 4) if only waters of hydration (i.e., water molecules in the primary hydration spheres of the products and reactants) were involved. They proposed that the hydration behavior of acids in mixed solvents is "water structure making," which is quite different from the "water structure breaking" behavior of concentrated electrolyte solutions, although reasons for this claim were

not well defined.

From practical and fundamental points of view, water is arguably the most important component in a mixed solvent system. Therefore, a basic understanding of the fundamental physical, chemical and structural properties associated with liquid water (which include solvation phenomena and what is currently known about proton transfer) is required to develop a better understanding of how cosolvents affect proton transfer. This is because the composition and structure of the molecules contained within the liquid system will ultimately influence its behavior. Thus, these subjects are briefly reviewed, before the concepts of excited state proton transfer are developed.

Physico-Chemical Properties of Water

The dynamic properties of liquid water ultimately depend upon the structure and properties of its individual molecules. Spectroscopic analysis of water in the gas phase has shown that water molecules are not linear. An angle of $105^{\circ}03'$ exists between the H-O-H bonds, while the internuclear O-H and H-H distances are $0.9568 \text{ \AA}$ and $1.54 \text{ \AA}$, respectively (54). In addition, dielectric investigations of water in the gas phase indicate that the isolated water molecule has a permanent electric dipole moment of 1.84 Debye (76). These observations do not yield a complete

picture of the isolated water molecule and its accompanying electronic charge distribution. However, with the aid of electrostatic models, molecular orbital theory, electron cloud distribution, wave functions and the calculation of physical properties, such as those described above, more precise descriptions of liquid water can be obtained. Perhaps the most reliable picture of water is Bjerrum's quadrupole model (77) in which eight ordered electrons move along four elliptical orbitals with two electrons in each orbital. The lobes of positive charge are directed along the O-H axis and are located primarily in the region of the two protons. The lobes of negative charge, essentially the two lone pair orbitals of the oxygen, are directed above and below the plane which contains both the oxygen and hydrogen nuclei. Conceptually, water takes the shape of a tetrahedron with its positive and negative charges being directed towards the apices of this tetrahedron (78).

The unusual tetrahedral configuration of the isolated water molecule (each oxygen atom $\sim sp^3$ hybridized) provides liquid water with its unique ability to form four hydrogen bonds. The two hydrogens of liquid water are capable of donating hydrogen bonds, while each of the two lone pair electrons of the oxygen atom are capable of accepting hydrogen bonds (79). It has been suggested that the anomalous properties of liquid water, such as the maximum

density at 4°C, the high dielectric constant, heat capacity, viscosity, thermal conductivity and critical temperature, may arise from the covalent characteristics of these hydrogen bonds (80).

The structure of liquid water, that is, the relative positions and motions of its individual molecules, has been scrutinized for decades. The first major breakthrough came in 1933, when Bernal and Fowler (78) inferred that a tetrahedral grouping (oxygen located at the center of a tetrahedron formed by four oxygen atoms, each 2.76 Å away) frequently occurs in the liquid as each water molecule is hydrogen bonded to its four nearest neighbors. In support, they cited that the structure of ice was partially retained upon melting and that within small regions of the liquid, water monomers remained in a lattice-like order. Investigations of the radial distribution functions of water with x-ray diffraction techniques have confirmed this conclusion (76,81).

Since that time, various model systems have been developed (for reviews see references 54,82-84). The most popular are based upon the existence of structured water as is readily seen in the "flickering cluster" model developed by Frank and Wen (80). They reasoned that highly directional cooperative covalent hydrogen bonds lead to the formation and dissolution of 3-dimensional hydrogen-bonded water

clusters. Coexisting with the ice-like, short-lived clusters which form and melt as a consequence of local energy fluctuations is a dense fluid made up of nonhydrogen bonded water molecules. According to Frank and Wen, the dissolution and formation of clusters is the rate-limiting step in the structural rearrangement of water. This is because perturbations of the liquid water system, which increased the population of hydrogen bonded molecules, would rigidify the structure of water on the subnanosecond time scale. On the other hand, any perturbation which would decrease hydrogen bond formation would ultimately loosen water structure by increasing the population of individual nonhydrogen bonded water molecules. The dielectric relaxation time ($\sim 10^{-11}$ sec) for liquid water (considered to be the lifetime of a hydrogen bond) (53) was cited as support for the short-lived clusters. Further, the uniform activation energies (4.6 kcal/mole) for dielectric relaxation (53), viscous flow (4.8 kcal/mole) and self-diffusion (4.6 kcal/mole) (85,86), which correspond roughly to the energy required to break and form hydrogen bonds, provide additional support to the idea that clustering was the rate limiting step in the structural rearrangement of water. However, in a review of the relaxation processes of water, Davis and Jarzynski (87) provide evidence which clearly shows that the rate determining step in water's

structural rearrangement is not clustering, but the jumping of individual water molecules from their temporary equilibrium positions. Thus, Frank and Wen's "flickering cluster" model and, for that matter any structural model of liquid water, is at best an approximation of reality. For a more accurate model, it becomes necessary to consider the relative positions and motions of water's individual molecules that arise in the process of thermal motion. According to Eisenburg and Kauzmann (54), the characteristic features of liquid water arise from two types of thermal motions. The first is a rapid oscillation which occurs about a temporal equilibrium position generally within a time-frame of about 10^{-13} sec. The second is a much slower displacement of the equilibrium position itself which occurs within a time frame of approximately 10^{-11} sec at 0°C. Unfortunately, current experimental techniques are not sensitive enough to provide the necessary details of water's momentary structure. Instead, one obtains an averaged picture of the immediate surroundings around an arbitrarily chosen water molecule during a time period which includes about a thousand molecular oscillations around a temporary equilibrium position. To circumvent this limitation, theoretical models like the Monte-Carlo (13,88,89) and molecular dynamics approach (14,90,91) to liquid water have been developed which tend to coincide with what has been

obtained experimentally. Consequently, water can be pictured as a statistically ordered three dimensional network of water molecules with different degrees of cooperative hydrogen bonding which are ordered in a tetrahedral fashion, complete with structural defects or vacancies.

Solvation

The process of solvation, where individually dissolved solute molecules become encapsulated by a coordination shell of more or less tightly bound solvent molecules, follows the addition of a solute to water. The magnitude of structural change that accompanies this process is not only dependent upon solute size, but also upon the degree and types of interactions which can occur between the solute and solvent molecules. For example, interactions may be solute-solute, solute-solvent or solvent-solvent in nature and may involve the so-called directional, induction and dispersion forces which are non-specific and unsaturable (i.e., ion-dipole, dipole-dipole, dipole-induced dipole or instantaneous dipole-induced dipole forces), or may involve the highly directional, saturating forces which can lead to stoichiometric molecular compounds (i.e., hydrogen bonds, charge transfer and electron-pair donor acceptor forces). In addition, solvophobic forces may play a role (92). Thus, the process of solvation is extremely complicated.

Current knowledge of solvation stems primarily from

fundamental thermodynamics. The energy changes that accompany this process are normally depicted by enthalpy characteristics, while any structural changes that occur are normally depicted by entropy characteristics. When combined, these changes constitute the change in Gibbs free energy of solvation, and therefore are representative of the chemical nature of the solution. They are interrelated through the following relationship:

$$\Delta G^{\circ}_{\text{Solv}} = \Delta H^{\circ}_{\text{Solv}} - T\Delta S^{\circ}_{\text{Solv}} \quad (1.11)$$

Daudel (93) claims that the measure of a solvent's solvation ability (i.e., $\Delta G^{\circ}_{\text{Solv}}$) is directly related to four distinct energy components that arise within the particular solvent upon solute dissolution. These components are:

- 1) The cavitation energy or the energy required to make a hole in the solvent system upon solute dissolution.
- 2) The orientation energy or the energy which arises from the partial orientation of the dipolar solvent molecules while in the presence of solvated solute molecules.
- 3) The isotropic interaction energy which results from such intermolecular forces as the electrostatic, polarization and dispersion energies.
- 4) The anisotropic interaction energy or the energy that accompanies specific interactions such as hydrogen

bonding or electron-pair donor acceptor bonds at well localized points in the dissolved molecules.

Unfortunately, a measure of solvation energetics from the Gibbs energy of solvation is not directly obtainable as it involves the energy change associated with the transfer of a solute from the gas phase into a solvent (93). However, it can be calculated with theoretical models (for reviews, see references 23,95-97). It is frequently replaced by the Gibbs free energy of transfer (ΔG_t°), which is a measure of the change in energy associated with the transfer of a solute from water (the reference solvent) to another solvent system (98,99).

Hydrocarbons

It has been suggested that the abnormal thermodynamic properties that arise when hydrocarbons are added to water, can be ascribed to an increase in the structuring of water (100,101). The behavior of the tetraalkylammonium halides and long chain organic salts in water, also appear to be related to this phenomenon (80).

The large positive molar heat capacities and the large negative heats and entropies of solution observed for nonelectrolytes in water have been attributed to the formation of water cages (100) or icebergs (101) around the solute molecules. Frank and Wen (80) claim that nonpolar solutes stabilize the ice-like clusters of water by

protecting them against the local energy fluctuations as pictured in their "flickering cluster" model. Némethy and Scheraga (102) suggest that nonpolar groups or molecules act as a fifth coordination neighbor to the tetrahedrally hydrogen-bonded water molecules. Thus, the weak Van der Waals forces which accompany such processes lower the energy of the associated water molecules, thereby stabilizing the cluster.

Monohydroxylic Alcohols

When placed in water, the monohydroxylic alcohols, tend to possess characteristics which are similar to hydrocarbons because of their apolar groups. They show large negative entropies and large positive molar heat capacities of solution (103,104) and their negative partial molar volumes in water increase with temperature and molecular size (105). Further, the enthalpies of transfer of alcohols from H_2O to D_2O , suggest that the apolar groups of the alcohols structure water (106). Support for these concepts can be found from the X-ray diffraction studies of ethanol (107) and t-butyl alcohol (108) in water. Results indicate that the ability of alcohols to structure water approaches a maxima when the alcohol mole-fraction is approximately 10-12 %. Any additional alcohol, results in the disruption of water structure; probably by breaking hydrogen bonds.

In addition to their nonpolar group, the monohydroxylic

alcohols possess a polar functional grouping ($-OH$) which adds to their rather unique properties in water. Unlike nonpolar molecules which tend to be forced out of solution by virtue of their large negative entropies of hydration, alcohols (at least the smaller ones) tend to remain in solution (109). Further, their ability to form cooperative hydrogen bonds leads to nonplanar structural arrangements of alcohol molecules (110).

At one time it was felt that alcohol dimers dominated the liquid structure of alcohols. However, Tucker and Christian (111) have found that dimers are relatively unimportant, even at low concentrations. Instead, evidence now suggests that tetramers (112) and/or pentamers (113) dominate their liquid structures. In fact, networks of cooperative hydrogen-bonded alcohols may even exist (113-115).

Recently, Krestov (116) has proposed that three distinct structural regions exist in mixed aqueous-alcohol solvent systems. In regions of high water concentration, alcohol molecules can be seen as preferentially filling the voids and interstitial spaces of water. Any displaced water becomes incorporated into the ice-like network (117,118). Thus, alcohols become incorporated into the tetrahedral structuring of water, at least up to the concentration of alcohol which correlates with the maximum stabilization of

water structure (119). However, with continued addition of alcohol to water, solvent structure in dynamic equilibrium goes from predominately water in nature to predominately alcohol in nature. Finally, at high concentrations of alcohol, there appears to be interstitial insertion of water molecules into the networks of alcohol structure (120), which tends to preserve the structuring of alcohols over a wide range of concentration (120,121).

Ions

In the solvation of ions in water, where electrostatic forces dominate, Frank and Wen (80), have considered that three distinct regions of solvent exist. The first of these regions consists of an ion surrounded by a coordination sphere of strongly bound solvent molecules which tend to be less mobile than the bulk solvent. At a distance well removed from this centralized ion, bulk solvent exists in its normal state which leaves an intermediate region of highly mobile, disordered solvent molecules. In this way, they were able to explain the "structure making" and "structure breaking" properties of ions of different charge and size in solution. Gurney (122), elaborated on the concept of different regions around the dissolved ion when he introduced the term cosphere for the zone surrounding a spherical ion in which significant differences in structure and properties are to be expected. Samoilov (123) found that

in some instances, the water molecules which surround a dissolved ion are more mobile than those found in the bulk. This is in contrast to the ordinary positive hydration of small spherical ions, which possess structure making effects on the solvent. This helped to explain the observation that aqueous solutions of certain salts (i.e., KI) are more fluid than pure water. It was suggested that when the exchange frequency of water molecules in the solvent shell is greater than that which occurs in the bulk, negative hydration occurs. Such ideas support the structure breaking effect of large singly-charged spherical ions on solvent molecules proposed earlier by Frank and Wen.

Relaxation Processes

The addition of solutes to water has been shown to rigidify water structure on the nanosecond time scale (80). Further, this phenomenon is reflected in the characteristic relaxation times of water (87).

Dielectric studies show that water molecules are able to respond to alternating electric fields up to very high frequencies of about 10^{10} sec^{-1} , and in the literature two types of dielectric relaxational times (τ_D and τ_L) have been discussed (124). The Debye relaxation time, τ_D , which is a phenomenological time constant related to the reorientational time constant of the solvent dipole, and the longitudinal relaxational time, τ_L , which is the time scale

for excited state solvation of a Debye solvent as well as the time scale for both ground and excited state solvation of dipolar and ionic solutes (125).

In pure liquid water, Pottel (126) states that small reversible shifts and rotations of atoms and molecules away from temporary equilibrium positions around which oscillations are possible relate to τ_L , while displacement (or irreversible rotations) relate to τ_D . According to Onsager (127), relaxational processes associated with solvation start far from the probe (i.e., in the bulk solvent and approximate the τ_L timeframe) and proceed inward towards the probe where the relaxation time approximates τ_D - - the "inverted snowball" effect. However, he later points out that a continuum of relaxation times between τ_D and τ_L is possible (128,129).

Recently, Robinson and coworkers (48) claimed that to accommodate the altered polarity that accompanies the ionization of weak acids, water molecules must rearrange themselves around the conjugate base in the τ_L time frame. In their defense, they cite that that the solvation dynamics of large polar molecules correlate well with the longitudinal relaxational times of alcohol molecules (130). Similar behavior has now been found in polar aprotic solvents (131). On the other hand, to solvate protons, Robinson and coworkers have envisioned that water molecules

must physically wrap themselves around protons, and that such motion can only occur on the τ_D time scale, leading to their backward sounding concept that "small elementary ions hydrate more slowly than larger ions." In defense, they cite their recent work in mixed solvents which spans the gap between a diffusion-controlled regime (translation of water molecules to satisfy the local concentration requirement) and a hydration-controlled regime (rotational diffusion of water molecules to satisfy the local structural requirement) (48).

It is readily seen that relaxational processes provides additional insight into the mechanism of solvation. However, the structural role played by the surrounding solvent remains open to debate.

Proton Transfer

The transfer of protons between molecules is one of the most important reactions found in chemistry today (132). It has been suggested that the kinetics associated with proton transfer are directly influenced by both the molecular dynamics and the molecular mechanics of the accompanying proton solvation (23). However, as in solvation, the molecular nature of these reactions remains unclear.

From Eigen's (37-39,62,66) theory of proton transfer, the solvated proton has been described in terms of the

following structural species:

- 1) the hydronium ion, H_3O^+ (primary solvation)
- 2) the complex ion, H_3O_4^+ (secondary solvation)
- 3) the hydrated complex ion (tertiary solvation)

The existence of hydronium and the complex ions have previously been demonstrated through mass spectroscopy (133,134). Further, neutron scattering (135) has led to the characterization of the hydronium ion (pyramidal in shape, with its oxygen atom residing $0.32 \text{ \AA}$ above the plane of the three protons, its H-O-H angles were 110.4° and its O-H internuclear distances were $1.01 \text{ \AA}$ in length). This gives strength to Eigen's claim that the excess positive charge on the hydronium ion was equally distributed between the three protons.

According to Eigen, the spatial characteristics of the hydronium ion provide it with its unique ability to form stable hydrogen bonds with neighboring water molecules which in turn leads to the formation of stable H_3O_4^+ complex ions. Further, it is Eigen's belief that the formation and destruction of additional, relatively labile hydrogen bonds along the border of the stable H_3O_4^+ complex ion leads to its "structural diffusion" in water. He claims that this is the manner in which rapid proton transfer takes place, the rate limiting being structural diffusion or the rate at which molecules along the periphery of the hydrated complex ion

came into positions to allow hydrogen bond formation and disruption. Unfortunately, experimental evidence does not fully support Eigen's proton transfer mechanism. Moreover, traditional theories of proton transfer which dated back to 1905, have relied on the Grotthuss "chain" mechanism to qualitatively explain prototropic behavior in solution.

In this classical mechanism, protons are considered to be transferred along a linear chain of hydrogen-bonded water molecules. For successive proton transfers to occur, each "proton jump" has to be followed by a structural rearrangement of the surrounding water molecules. Conway et al. (23, 136) suggest that the transfer of a proton from a hydronium ion to an adjacent water molecule leads to an unfavorable orientation of neighboring water molecules. They claimed that classical proton transfer mechanisms were too slow and that proton tunneling across hydrogen bonds was too fast to account for proton mobilities. Thus, the rate-limiting step in proton transfer must be the rotation of hydrogen-bonded water molecules near the H_3O^+ ion, i.e., rotation of molecules within the immediate proton hydration sphere. They also found that this type of mechanism could account for hydroxide's mobility in water.

According to Erdey-Gruz and Lengyel (138), different mechanisms for proton transfer must be assumed because the proton transfer process depends both on the nature and

composition of the solvent system. In support, Robinson et al. (48), have recently shown that in aqueous-alcohol solvent systems, the mechanism of proton transfer spans the gap between a diffusion-controlled regime (Eigen's model) and a hydration-controlled regime (Conway et al.'s model). They also found that acid dissociation and its attendant proton hydration, produced the H_3O_4^+ ion as its direct kinetic product. This tends to substantiate Eigen's claim that rapid proton transfer occurs within a strongly hydrated complex. Further, Castleman and coworkers (137) have now provided direct experimental evidence of stable cluster ions through vacuum-UV photoionization. However, additional investigations, preferentially in solution, are still required to substantiate Eigen's claim.

Proton Transfer in the Excited Singlet State

Excited state proton transfer, a special form of proton transfer exhibited by certain aromatic acids and bases following photon absorption, will be used as a tool to probe solvation phenomenon and solvent structure in mixed aqueous solvent systems. The kinetics associated with such processes, which occur during the lifetime (typically 10^{-7} to 10^{-11} s) of the lowest excited singlet state (S_1), can be determined with steady-state and time-resolved fluorometric techniques. The underlying principles associated with each

of these techniques will be preceded by a brief discussion on the electronic and accompanying spectral effects of proton transfer in electronically excited singlet states.

Electronic and Accompanying Spectral Effects

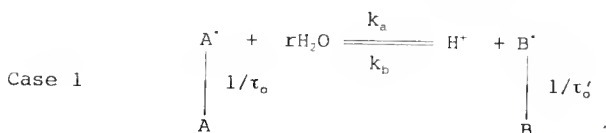
In 1949, Förster (139) concluded that the spectral dependence on pH of electronically excited aromatic acids and bases, which was initially observed by Weber (140), arose from proton transfer processes that took place during the lifetime of the excited state. This behavior was subsequently quantified by Weller (141) with his steady-state kinetic approach to these dynamic processes. Several advances have stemmed from these pioneering efforts. For example, it is now recognized that aromatic molecules possessing functional groups which donate electronic charge to the aromatic system become stronger acids in the excited state because the electrostatic attraction between the proton and the functional group decreases, ($pK_a' < pK_a$). Hydroxyl, sulfhydryl, and amino groups along with pyrrolic nitrogens all donate electronic charge. On the other hand, aromatic molecules possessing functional groups with vacant low lying π orbitals accept electronic charge from the aromatic system upon excitation, making proton abstraction more difficult, ($pK_a' > pK_a$). Thus, aromatic compounds of this type become stronger bases in the excited state. Carbonyl, carboxyl, carboxylate, and amide groups are

capable of accepting electronic charge. In addition, pyridine nitrogen atoms (such as those found in quinoline) show increased basicity upon excitation even though they do not possess vacant low lying π orbitals because electronic charge becomes localized on the nitrogen atom. Further, the absorption and fluorescence spectra of aromatic molecules capable of transferring protons in the excited state will reflect these changes. For example, upon excitation the longest wavelength absorption and fluorescence bands of aromatic molecules which possess electron donor groups shift to shorter wavelengths upon protonation and to longer wavelengths upon dissociation. Such behavior is indicative of a decrease in basicity and an increase in acidity upon going from the ground to excited states. Similarly, the long-wavelength absorption and fluorescence bands of aromatic molecules possessing electron acceptor groups shift to longer wavelengths upon protonation and shorter wavelengths upon dissociation. This results from an increase in the basicity and a decrease in the acidity of such molecules upon going from ground to excited state.

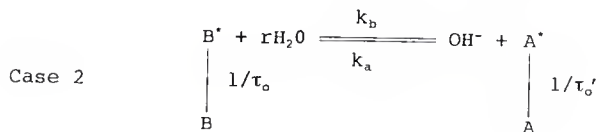
Steady-State Kinetics

The approach developed by Weller (141) to determine the excited state proton transfer rate constants is based upon the pH dependent fluorescent intensities of the molecular species involved and the assumption that all photophysical

and photochemical processes deactivating S_1 have achieved steady-state. With this in mind, proton transfer from excited aromatic acids and bases in dilute aqueous solutions can be considered to occur by one of two simple reversible two-state reaction schemes, shown below. Of course, more complex behavior is often observed, but Weller's simple model systems provide the conceptual framework for the more complex processes. In the first case, which describes the excited state dissociation of 2-,3- and 4-hydroxybiphenyl, weak aromatic acids examined in this investigation, water acts as the proton acceptor while the solvated hydroxybiphenyl(s) act as the proton donor.



The second case describes the excited state reaction of 6-methoxyquinoline, a weak base that was examined in this investigation. Here, water acts as the proton donor and the solvated 6-methoxyquinoline as the proton acceptor.



In both cases, r represents the difference between the number of water molecules solvating both reactants and products. In turn, τ_0 and τ_0' represent the lifetimes of the reactants and products in S_1 in the absence of proton transfer, which are equal to the reciprocals of the rate constants for photophysical deactivation of S_1 are given as:

$$\tau_0 = \frac{1}{(k_f + k_d)} \quad \text{and} \quad \tau_0' = \frac{1}{(k_f' + k_d')}$$

where k_f and k_f' are the fluorescence rate constants and k_d and k_d' are the rate constants for radiationless deactivation.

From Case I, the relative quantum yields of fluorescence (ϕ/ϕ_0 and ϕ'/ϕ_0') for the excited acid (A^*) and conjugate base (B^*) respectively, are obtained by simultaneously solving the following two equations for the disappearance of A^* and B^* from S_1 :

$$-\int_{t_i}^0 d[A^*] = \int_0^\infty \left(\frac{1}{\tau_0} + k_a \right) [A^*] dt - \int_0^\infty k_b [H^*] [B^*] dt \quad (1.12)$$

$$-\int_{t_i}^0 d[B^*] = \int_0^\infty \left(\frac{1}{\tau_0'} + k_b [H^*] \right) [B^*] dt - \int_0^\infty k_a [A^*] dt \quad (1.13)$$

In the equations above, $[A^*]$ and $[B^*]$ are the respective relative probabilities of finding an A^* and a B^* molecule in the excited state at any time (t) following excitation. The rate constants (or probabilities) of dissociation and

protonation of A^* and B^* are k_a and k_b , respectively, and $[H^+]$ is the molar concentration of hydrogen ion. The lower limits of integration, α_A and α_B , are the fractions of excited A and B or the fractions of total light energy absorbed by A and B at the wavelength of excitation, respectively, and are defined as follow:

$$\alpha_A = \frac{\epsilon_A[A]}{\epsilon_A[A] + \epsilon_B[B]} \quad \text{and} \quad \alpha_B = \frac{\epsilon_B[B]}{\epsilon_A[A] + \epsilon_B[B]}$$

where the molar absorptivities of A and B at the excitation wavelengths are given by ϵ_A and ϵ_B . The ground state concentrations of A and B at equilibrium and at known pH are $[A]$ and $[B]$. In turn, the relative quantum yields of fluorescence are defined by the integrals (141):

$$\int_0^\infty [A^*] dt = \tau = \frac{\Phi}{\Phi_0} \tau_0 \quad \text{and} \quad \int_0^\infty [B^*] dt = \tau' = \frac{\Phi'}{\Phi_0} \tau'_0$$

where the actual lifetimes of A^* and B^* are τ and τ' and are proportional to relative fluorescent intensity measurements when an isosbestic point ($\epsilon_A = \epsilon_B$) is used for excitation. Integration and rearrangement of equations (1.12) and (1.13) gives:

$$\frac{\Phi}{\Phi_0} = \frac{\alpha_A + k_b \tau'_0 [H^*]}{1 + k_a \tau_0 + k_b \tau'_0 [H^*]} \quad (1.14)$$

$$\frac{\phi'}{\phi_0} = \frac{\alpha_B + k_a \tau_0}{1 + k_a \tau_0 + k_b \tau_0' [H^*]} \quad (1.15)$$

based upon the relationship that the fractions of directly excited A and B are equal to unity, ($\alpha_A + \alpha_B = 1$). Equations (1.14) and (1.15) can then be combined to give:

$$\frac{\phi/\phi_0}{\phi'/\phi_0' - \alpha_B} = \frac{1}{k_a \tau_0} + \frac{k_b \tau_0' [H^*]}{k_a \tau_0} \left(\frac{\phi'/\phi_0'}{\phi'/\phi_0' - \alpha_B} \right) \quad (1.16)$$

where it is readily seen, that a plot of:

$$\frac{\phi/\phi_0}{\phi'/\phi_0' - \alpha_B} \quad \text{vs.} \quad [H^*] \frac{\phi'/\phi_0'}{\phi'/\phi_0' - \alpha_B}$$

provides a means by which to calculate k_a and k_b (the rate constants of proton exchange) provided the lifetimes of the species in the excited state are known.

When protonation of the excited conjugate base occurs at $[H^+] \gg K_a$, where K_a is the ground state dissociation constant, $\alpha_B = 0$ and equation (1.16) reduces to Weller's equation (141).

$$\frac{\phi/\phi_0}{\phi'/\phi_0'} = \frac{1}{k_a \tau_0} + \frac{k_b \tau_0'}{k_a \tau_0} [H^*] \quad (1.17)$$

Under these conditions, the excited state reaction is reversible. However, at $\text{pH} > 4$ ($[H^+] \leq 1 \times 10^{-4} \text{M}$) the reaction

is virtually independent of pH and irreversible. This is because k_b is diffusion controlled ($k_b \leq 5 \times 10^{10} \text{M}^{-1}\text{s}^{-1}$) and τ_0' is approximately $1 \times 10^{-8} \text{s}$, making $k_b \tau_0' [\text{H}^+] \leq 5 \times 10^{-2}$. Under these conditions, equation (1.17) to be reduced to:

$$\frac{\phi'/\phi_0'}{\phi/\phi_0} = k_a \tau_0 \quad (1.18)$$

which provides a means to directly determine k_a , provided τ_0 is known.

From Case 2, the relative quantum yields of fluorescence (ϕ/ϕ_0 and ϕ'/ϕ_0') for the excited base (B^*) and conjugate acid (A^*) respectively, are obtained by simultaneously solving the following steady-state equations for the disappearance of B^* and A^* from S_1 :

$$-\int_{\mu_s}^0 d[B^*] = \int_0^\infty \left(\frac{1}{\tau_0} + k_b \right) [B^*] dt - \int_0^\infty k_a [OH^-] [A^*] dt \quad (1.19)$$

$$-\int_{\mu_a}^0 d[A^*] = \int_0^\infty \left(\frac{1}{\tau_0} + k_a [OH^-] \right) [A^*] dt - \int_0^\infty k_b [B^*] dt \quad (1.20)$$

As in Case 1, the excited state rate constants, k_a and k_b , can be determined. Integration and rearrangement of equations (1.19) and (1.20) gives the following equations:

$$\frac{\phi}{\phi_0} = \frac{\alpha_B + k_a \tau_0' [OH^-]}{1 + k_b \tau_0 + k_a \tau_0' [OH^-]} \quad (1.21)$$

$$\frac{\phi'}{\phi_0} = \frac{\alpha_A + k_b \tau_0}{1 + k_b \tau_0 + k_a \tau_0 [\text{OH}^-]} \quad (1.22)$$

which when combined leads to the following complex expression:

$$\frac{\phi/\phi_0}{\phi'/\phi'_0 - \alpha_A} = \frac{1}{k_b \tau_0} + \frac{k_a \tau'_0 [\text{OH}^-]}{k_b \tau_0} \left(\frac{\phi'/\phi'_0}{\phi'/\phi'_0 - \alpha_A} \right) \quad (1.23)$$

which can be reduced, when $[\text{H}^+] \ll K_a$, $\alpha_A = 0$, to another one of Weller's reversible two-state kinetic equations (142):

$$\frac{\phi/\phi_0}{\phi'/\phi'_0} = \frac{1}{k_b \tau_0} + \frac{k_a \tau'_0 [\text{OH}^-]}{k_b \tau_0} \quad (1.24)$$

which provides the means to calculate the individual rate constants, provided appropriate lifetimes are known. The relative fluorescence intensities and their ratios become irreversible and independent of pH when $\text{pH} < 9$, because $k_a \tau'_0 [\text{OH}^-] \ll 1$. Under these conditions, equation (1.24) reduces to:

$$\frac{\phi'/\phi'_0}{\phi/\phi_0} = k_b \tau_0 \quad (1.25)$$

which allows direct determination of k_b , provided τ_0 is known.

It is important to recognize that the preceding steady-state development of excited state proton transfer is based upon the assumption that the presence of solute molecules or

ions in solution does not affect the properties of the reactants or the activated complex. However, when ions are present, deviations from ideality become apparent even at low concentrations. Thus, the observed rate constants, as defined in equations (1.14), (1.15), (1.21) and (1.22), are not necessarily those which would be obtained at infinite dilution. According to Brønsted (143,144), the medium effects on the rate constants in relatively dilute solutions of acid and base within the pH range of 1-11, can be compensated for by using the kinetic activity factor (F), where F is of the form:

$$F = \exp\left(\frac{Z_1 Z_2 e^2}{\epsilon k T a} \cdot \frac{\kappa}{1 + \kappa}\right) \quad (1.26)$$

where $Z_1 e$ and $Z_2 e$ are the charges of the ionic reactants, ϵ is the dielectric constant, kT is the Boltzmann's factor, a is the ionic encounter distance and κ is the reciprocal thickness of the ionic atmosphere. When compensation is made, equations (1.16) and (1.23) take on the following form:

$$\frac{\phi/\phi_0}{\phi'/\phi'_0 - \alpha_B} = \frac{1}{K_B \tau_0} + \frac{K_B \tau'_0 [H^+]}{K_A \tau_0} F \left(\frac{\phi'/\phi'_0}{\phi'/\phi'_0 - \alpha_B} \right) \quad (1.27)$$

$$\frac{\phi/\phi_0}{\phi'/\phi'_0 - \alpha_A} = \frac{1}{K_B \tau_0} + \frac{K_A \tau'_0 [OH^-]}{K_B \tau_0} F \left(\frac{\phi'/\phi'_0}{\phi'/\phi'_0 - \alpha_A} \right) \quad (1.28)$$

Perhaps the single most important advance in the study of excited state proton transfer processes since Weller developed the steady-state equations has been the development of sensitive detection methods such as pulsed and phase-shift demodulation fluorimetry (145). With these techniques it is now possible to look directly into the reaction process by monitoring the fluorescence decay of the sample (146). The pulsed method that was employed in this investigation was time-correlated single photon counting (147). The theory associated with the determination of kinetic rate constants with this method are elaborated upon below. However, the principles behind time-resolved measurements is saved for the experimental section.

Time-resolved Kinetics

According to Laws and Brand (148), excited state proton transfer rate constants can be evaluated using pulsed methods, provided the following assumptions are made. First, the excited state molecule exists in two states, each with a distinct emission spectrum and second, that the kinetic rate constants of these states are independent of the emission wavelength.

In the first case, which describes excited state dissociation of 2-,3- and 4-hydroxybiphenyl, the following differential equations apply:

$$-\frac{d[A^*]}{dt} = \left(\frac{1}{\tau_0} + k_a \right) [A^*] - k_b [B^*] [H^*] \quad (1.29)$$

$$-\frac{d[B^*]}{dt} = \left(\frac{1}{\tau_0} + k_b [H^*] \right) [B^*] - k_a [A^*] \quad (1.30)$$

Boundary conditions are chosen such that $[A^*] = [A^*_0]$ and $[B^*] = 0$ at $t = 0$ (148,149). Following δ -pulse excitation, the time-dependent intensities of A^* and B^* at any wave number $\bar{\nu}$ are given by:

$$I_A(\bar{\nu}, t) = \alpha_1(\bar{\nu}) \exp^{-t/\tau_1} + \alpha_2(\bar{\nu}) \exp^{-t/\tau_2} \quad (1.31)$$

$$I_B(\bar{\nu}, t) = \beta_1(\bar{\nu}) \exp^{-t/\tau_1} + \beta_2(\bar{\nu}) \exp^{-t/\tau_2} \quad (1.32)$$

The decay times of A^* and B^* will be given by:

$$\tau_1^{-1}, \tau_2^{-1} = \frac{1}{2} \left[(\gamma_A + \gamma_B) \pm ((\gamma_B - \gamma_A)^2 + 4k_a k_b [H^*])^{1/2} \right] \quad (1.33)$$

when γ_A and γ_B are defined as follows:

$$\gamma_A = \frac{1}{\tau_0} + k_a \quad \text{and} \quad \gamma_B = \frac{1}{\tau_0} + k_b [H^*]$$

The pre-exponential factors in equations (1.31) and (1.32) are given by:

$$\alpha_1(\bar{\nu}) = C_A(\bar{\nu}) [A_0^*] \frac{\gamma_A - \gamma_2}{\gamma_1 - \gamma_2} k_{fA} \quad (1.34)$$

$$\alpha_2(\bar{\nu}) = C_A(\bar{\nu}) [A_0^*] \frac{\gamma_1 - \gamma_A}{\gamma_1 - \gamma_2} k_{fA} \quad (1.35)$$

$$-\beta_1(\bar{\nu}) = \beta_2(\bar{\nu}) = \frac{C_B(\bar{\nu}) k_a [A_0^*] k_{fB}}{\gamma_1 - \gamma_2} \quad (1.36)$$

In the equations above, $\gamma_1 = \tau_1^{-1}$, $\gamma_2 = \tau_2^{-1}$, k_{fA} and k_{fB} are the probabilities of radiative deactivation, while $C_A(\bar{\nu})$ and $C_B(\bar{\nu})$ are the emission spectra of species A^* and B^* , respectively, normalized to unit area:

$$C_A(\bar{\nu}) = \frac{\theta_A(\bar{\nu})}{\int_0^\infty \theta_A(\bar{\nu}) d\bar{\nu}} \quad (1.37)$$

$$C_B(\bar{\nu}) = \frac{\theta_B(\bar{\nu})}{\int_0^\infty \theta_B(\bar{\nu}) d\bar{\nu}} \quad (1.38)$$

where $\theta_A(\bar{\nu})$ and $\theta_B(\bar{\nu})$ are the number of quanta emitted at any wavenumber by the A^* and B^* species, respectively.

At any given wavenumber the time-resolved decay of

fluorescence is given by:

$$I(\bar{\nu}, t) = I_A(\bar{\nu}, t) + I_B(\bar{\nu}, t) \quad (1.39)$$

where the individual decays of A* and B* are given by equations (1.31) and (1.32), respectively, such that their substitution into the above equation leads to

$$I(\bar{\nu}, t) = (\alpha_1 + \beta_1)\exp^{-t/\tau_1} + (\alpha_2 + \beta_2)\exp^{-t/\tau_2} \quad (1.40)$$

In case 2, the equation for time-resolved decay is identical to the ones given above, provided one makes the appropriate corrections for the respective decay times and pre-exponential factors. These components are given as follows:

$$\tau_1^{-1}, \tau_2^{-1} = \frac{1}{2} [(\gamma_A + \gamma_B) \pm ((\gamma_B - \gamma_A)^2 + 4k_a k_b [OH^-])^{1/2}] \quad (1.41)$$

$$-\alpha_1(\bar{\nu}) = \alpha_2(\bar{\nu}) = C_A(\bar{\nu}) \frac{k_b [B_0^*] k_{fA}}{\gamma_1 - \gamma_2} \quad (1.42)$$

$$\beta_1(\bar{\nu}) = C_B(\bar{\nu}) [B_0^*] \frac{\gamma_B - \gamma_2}{\gamma_1 - \gamma_2} k_{fB} \quad (1.43)$$

$$\beta_2(\bar{\nu}) = C_B(\bar{\nu}) [B_0^*] \frac{\gamma_1 - \gamma_B}{\gamma_1 - \gamma_2} k_{fB} \quad (1.44)$$

These results are readily understood when the

differential kinetic equations of case 2, seen below, are compared with those of case 1.

$$-\frac{d[B^*]}{dt} = \left(\frac{1}{\tau_0} + k_b \right) [B^*] - k_a [A^*][OH^-] \quad (1.45)$$

$$-\frac{d[A^*]}{dt} = \left(\frac{1}{\tau_0} + k_a[OH^-] \right) [A^*] - k_b[B^*] \quad (1.46)$$

Returning to equation (1.40), one can see that at any given wavelength the fluorescence decay appears to be biexponential. It should be pointed out, however, that both the decay times and pre-exponential factors are actually complex functions of the kinetic constants of the system. Further, the pre-exponential factors are also functions of the spectral distributions of the species present. Therefore, one must not make the assumption that the decay times obtained from this expression represent unique components of the sample system unless other indications are available to support this interpretation.

There are several techniques by which rate parameters can be extracted from fluorescent decay measurements (145,150). In this investigation, the "difference of lifetimes" method was used to extract the protonation and dissociation rate constants from 6MQ and 2HBP, respectively. The principles behind this technique are as follows. In case

1, if pH is adjusted such that $[B'] = 0$ and $k_s \rightarrow 0$, fluorescence decay becomes monoexponential:

$$I(\bar{\nu}, t)_A = I_A(\bar{\nu}, t = 0) \exp^{-\gamma_1 t} \quad (1.47)$$

where $\gamma_1 = 1/\tau_0$. Similarly, at neutral pH where $[H']$ and $k_0[H'] \rightarrow 0$, decay is also found to be monoexponential. However, under these conditions, $\gamma_1 = (1/\tau_0 + k_s)$. Thus, if τ_0 can be determined and it is independent of the concentrations of A and H^+ , the difference between the reciprocals of the two lifetimes yields the forward rate constant k_a . Finally, in alkali boundary conditions can be chosen such that $[B'] = [B'_0]$ and $[A'] = 0$ at $t = 0$. Under these conditions, the decay profile of the conjugate base is monoexponential, and the following equation applies:

$$I(\bar{\nu}, t)_B = I_B(\bar{\nu}, t = 0) \exp^{-\gamma_2 t} \quad (1.48)$$

where $\gamma_2 = 1/\tau'_0$, provided τ'_0 is independent of B and OH^- . The use of this method provides a means by which the forward rate constant can be determined. It also serves as a means by which to obtain the lifetime of the individual species in the absence of proton transfer, thus allowing for the solution of the steady-state equations. The difference of lifetime method can be developed for Case II in a similar fashion. However, in the case of 6MQ, quenching of fluorescence prevented the direct measurement of τ_0 . An

additional set of experiments were required to estimate τ_0 before the difference method could be used to calculate the protonation constant for 6MQ. The rationale behind these experiments will be better understood if it is left for the results and discussion section.

Objectives

Recently Kelly (151), using traditional steady-state spectroscopic technique, claimed that the activity of water provides the best means through which the reduction in excited state proton transfer in mixed aqueous-organic solvent systems can be described. However, since his results were not conclusive, it was suggested that excited state proton transfer must be dependent upon some yet unidentified physical chemical property of the mixed solvent system, such as changes in the secondary or tertiary solvation of the functional group bearing the reactive proton. Ultimately, he could only conclude that additional investigations in mixed aqueous organic solvent systems would be required before a proton transfer model acceptable in all respects could be proposed.

The present study was, therefore, undertaken to further investigate and attempt to correlate the medium dependence of excited state proton transfer in mixed aqueous organic solvent systems with the activity of water in a manner

similar to that adopted by Schulman and Kelly (74,75). However, in this investigation, time-resolved spectroscopic technique will be combined with traditional steady-state techniques because the union of these techniques allows the rate constants of these elementary proton transfer reactions to be determined with greater accuracy than by either technique alone. Further, since traditional steady-state methods can only focus on the role of solute structure and reactivity in excited state proton transfer reactions, the direct observation of ionic dissociation processes in water with time-resolved spectroscopic methods should provide additional insight into the effects of both solute and solvent properties on the excited state proton transfer process.

The weak base, 6-methoxyquinoline (6MQ) and the weak acids, 2-, 3- and 4-hydroxybiphenyl (2HBP, 3HBP and 4HBP) depicted in figure 1.1, were used as excited state probes in the current mixed solvent investigations. It was felt that an examination of 6MQ in mixed solvents would help to determine if the steady-state approach developed by Schulman and Kelly with the weakly acidic, sulfonated naphthols (74,75) could be extended to weak bases. However, before these studies could be conducted, it was imperative that the behavior of 6MQ in aqueous media be properly identified to provide a much needed reference point from which to base the

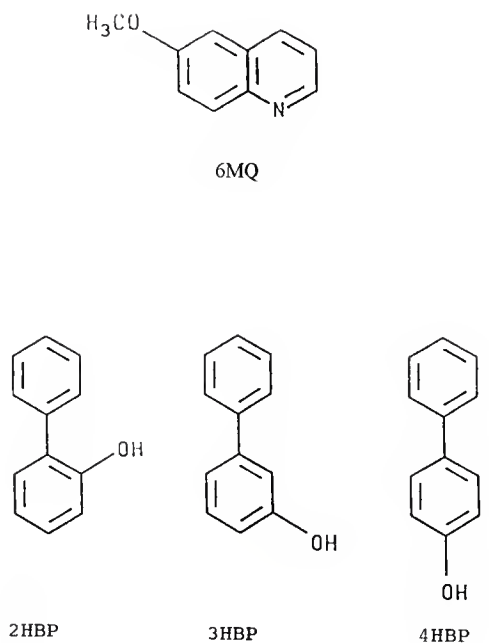


Figure 1.1. The chemical structures of 6-methoxyquinoline (6MQ), 2-hydroxybiphenyl, 3-hydroxybiphenyl and 4-hydroxybiphenyl (2, 3, and 4HBP).

prototropic behaviors of 6MQ in mixed aqueous-organic solvent systems. In addition, aqueous studies will hopefully clear up some conflicting reports in the literature on 6MQ. The major discrepancies have been listed in table 1.1, below.

Table 1.1. Acid-base properties of 6-methoxyquinoline in the lowest excited singlet (S_1) state.

k_b ($\times 10^{-9} \text{ s}^{-1}$)	k_a ($\times 10^{-10} \text{ M}^{-1} \text{ s}^{-1}$)	pK_a^*	Reference
0.64	2.6	10.4	152
2.0 ± 0.6	3.1 ± 0.6	10.8 ± 0.1	43
1.2	N.R.	N.R.	153

It was felt that an investigation of the hydroxybiphenyls, first, would provide a means to determine if the steady state approach developed by Schulman and Kelly could be extended to weak acids systems, which are composed of two chromophores that are coupled by a single carbon-carbon bond. Second, since a $42 \pm 2^\circ$ dihedral angle exists between the two rings of the biphenyl molecule in the ground state (154) since the molecule becomes planar upon excitation (155), it was felt that fluorometric investigations of these simple nonrigid acids may provide some additional insight into the behavior of larger nonrigid molecular systems. Finally, before an investigation can be conducted in mixed solvents, the aqueous properties of these

probes in the lowest excited state must be determined, because even though Bridges et al. (156) have found that the hydroxybiphenyls exhibit prototropic activity, they did not characterize this activity.

CHAPTER 2 EXPERIMENTAL

Materials

The probes, 2-, 3-, and 4-hydroxybiphenyl, were purchased from Aldrich Chemical Company, Inc., Milwaukee, Wis. The 2-isomer, 99+ % purity, was used as supplied. However, a triple recrystallization procedure from 10% ethanol was required to clean up the 3- and 4- isomers prior to use. The probe, 6-methoxyquinoline, was purchased from Pfaltz and Bauer, Inc., Flushing, N.Y. To remove contaminants, the compound was converted to its perchlorate salt in 90 % ethanol with the addition of 70% perchloric acid. The perchlorate salt was then recrystallized twice from 90 % ethanol solutions prior to use. The purities of these probes were subsequently confirmed when absorptiometric pKa determinations were found to be in agreement with the observed literature values (152,157).

Acetonitrile, dimethyl sulfoxide, formamide, dioxane, methanol, isopropanol, tertiary butanol, perchloric acid (70%), sulfuric acid (all reagent grade), sodium hydroxide (1.0 M and 5.0 M) (analytical grade), certified pH 4.0, 7.0

and 10.0 buffer solutions and Davison 4 Å molecular sieves were purchased from Fisher Scientific Co., Fairlawn N.J. Absolute ethanol was purchased from AAPER Alcohol and Chemical Co., Shelbyville, Ky. Boiled deionized water was used in the preparation of all aqueous solutions.

Instrumental

Absorption measurements were made on a Perkin Elmer Lambda-3 spectrophotometer. Absorption spectra on all probes were monitored from 500 nm to 210 nm.

Fluorescence measurements were made on a Perkin Elmer LS-5 fluorescence spectrophotometer whose monochrometers were calibrated against the xenon line emission spectrum. The wavelength variable spectral output was uncorrected for instrumental response. Excitation of the probes was effected at an isosbestic point found in the absorption spectra. For the 2-, 3- and 4-hydroxybiphenyl isomers, the excitation wavelengths were 290 nm, 284 nm, and 270 nm, respectively. For 6-methoxyquinoline, the excitation wavelength was 289 nm. In all cases, emission was monitored from 320 nm to 540 nm. Excitation and emission slits were set at 3 nm.

Emission decay measurements were made on a Photochemical Research Associates (PRA) model 1551 time-correlated, single photon counter. The excitation source was an H₂-filled spark gap (PRA model 510 B). The instrument

response was ≈ 2.0 ns fwhm. In all cases, excitation light was filtered with a Corion near-UV 10 nm bandpass filter that had a maximum transmittance at 280 nm. Emission light was filtered with a Schott UG-11 near-UV 10 nm bandpass filter that had a maximum transmittance at 350 nm or with Schoeffel UV-cutoff filters at 418 nm or 450 nm. The excitation repetition rate was 30 KHz and the sampling rate was ≤ 500 Hz. Analysis of emission decay data was carried out on an IBM-PC using a deconvolution program from PRA. A Fisher Accumet 950 pH meter which employed a Fisher pencil gel-filled silver/silver chloride combination electrode calibrated against pH certified buffer solutions was used for pH measurements. Gilmont pipets (0.02, 0.2 and 1.0 ml) were calibrated and employed to deliver volumetric amounts of water and cosolvent mixtures.

Methods

Steady-State Emission Spectroscopy

Aqueous acid-base titrations

A known volume of a 10^{-2} M ethanolic stock solution, prepared prior to investigation, was micropipetted into a series of 10 ml volumetric flasks. The aliquot was then evaporated under a stream of nitrogen gas before the residue was brought to volume by dilution to the mark with water, 0.01 M, 0.1 M, 1.0 M, 3.0 M, or 5.0 M standardized H_2SO_4 or

NaOH. The final probe concentration was $\sim 10^{-5}$ M, chosen to keep the absorbance at each of the excitation wavelengths to less than 0.02 absorption units, thereby reducing the probability of nonlinear fluorescence.

The aqueous acid-base titrations, preformed at room temperature, were conducted as follows:

- 1) Two milliliters of the aqueous probe solution was pipetted into a 1 cm² cuvette having a 4 ml volume, and its emission spectrum and pH were obtained.
- 2) The above solution was then titrated with an acidic probe solution. Fluorescence spectra were scanned and pH was measured after each increment of titrant was added. The titration was carried out until the excited state reaction could no longer be detected, (i.e., the fluorescence species either reached a maxima or went to zero, respectively). Calculation of ionic strength at each point in the titration curve was carried out using the formal concentration of the acid.
- 3) Steps 1-2 were repeated with alkaline probe solutions.
- 4) The relative fluorescence intensities for both the acidic and basic species of excited probe, as a function of pH, were converted to relative quantum yields for analysis. The relative quantum yields for the neutral species of the hydroxybiphenyl isomers or 6-methoxyquinoline were obtained with the following equation, $\Phi/\Phi_0 = [(F_N - F_C) / (F_N^0 - F_C)]_h$,

where F_N , F_N^0 , are the relative fluorescence intensities of the neutral species at intermediate and maximal pH values and F_c is the residual relative fluorescence intensity of the charged species, all at the analytical wavelength, λ , which is generally taken to be the wavelength at which the fluorescence of the individual species is maximum.

Aqueous-organic solvent titrations

Working solutions for investigation of the forward reaction were prepared from stock solution as above, with one exception. In this case, they were brought to final volume by either the addition of water or a dried organic solvent, which had been stored over 4 Å molecular sieves for a minimum of 24 hrs.

The mixed aqueous-organic solvent titrations for investigation of the forward reaction, performed at room temperature were conducted as follows:

- 1) Two milliliters of the aqueous probe solution was pipetted into a 1 cm² cuvette having a 4 ml volume and its fluorescence spectrum was recorded.
- 2) The above solution was then titrated with the organic probe solution. Fluorescence spectra were scanned after each increment of titrant was added until the mole fraction of the organic component approached unity, provided emission from the excited state reaction could still be detected.
- 3) The fluorescence spectrum of the non-aqueous solution was

also recorded.

4) The relative fluorescence intensity obtained for each species of probe as a function of the organic cosolvent mole fraction was converted to relative quantum yield in a fashion similar to above. However, rather than isolating the individual fluorescence maxima for each species of probe at each point in the mixed solvent titration, fluorescence maxima were fixed to the initial aqueous values. This normalization procedure provided a consistent means by which the effects of the individual solvents on the forward rate constants could be compared.

Aqueous-organic acid-base titrations

Solutions of the probe, 2-hydroxybiphenyl, were prepared by pipetting a known volume of dried organic cosolvent to three 10 ml volumetric flask, each of which contained probe such that the final concentration was 10^{-5} M. One of the 10 ml flasks was brought to volume with water. The remaining flasks were brought to volume with water after addition of a known aliquot of standardized H_2SO_4 or NaOH , respectively. Thus, each flasks contained the same concentration of organic cosolvent.

The mixed solvent acid-base titrations for the investigation of the back reaction, preformed at room temperature were conducted as follows:

1) Two milliliters of the aqueous-cosolvent mixture was

pipetted into a 4.0 ml cuvette and its fluorescence spectrum was recorded.

2) The above solution was then titrated with the acidic mixed solvent mixture. Fluorescence spectra were scanned after each increment of titrant was added, and the titration was carried out until the fluorescence of the conjugate base could no longer be observed and the fluorescence of the acid was maximal and constant. Calculation of ionic strength at each point of the titration curve was carried out using the formal concentration of the acid.

3) The fluorescence spectrum of the basic probe solution was subsequently obtained to allow for the generation of relative quantum yield.

4) The relative fluorescence intensity obtained for each species of probe, as a function of the formal hydrogen ion concentration in each of the mixed solvent systems investigated, was converted to relative quantum yield after the system was normalized. In the normalization procedure, the relative quantum yield of the conjugate base at the specific organic cosolvent mole fraction investigated was fixed to the relative quantum yield obtained in the previous titration. However, the relative quantum yield for the acid under identical conditions was obtained through the following relationship (141), $1 - \Phi'/\Phi_o = \Phi/\Phi_o$. The rationale for these procedures are given below.

Upon analysis, the sum of the observed relative quantum yield for both excited state species of probe did not add up to one. In fact, as organic concentrations were increased, the sum of the observed relative quantum yield from both species was found to deviate from 1 by as much as 40 %. Any calculation and comparison of kinetic rate constants under these conditions would have been meaningless. With further investigation, it was found that the neutral species undergoes extensive quenching in the acidic range as the concentration of the organic solvent is increased, due in part to the organic concentration and in part to the acid concentration. The conjugate base on the other hand did not exhibit any quenching processes. In fact, the relative quantum yield values obtained from both mixed solvent studies for the conjugate base were generally found to be within 3 % of each other. The effect of quenching by organic cosolvents was minimized when the relative quantum yield of the neutral species at each mole fraction investigated was fixed to the relative quantum yield of the conjugate base as noted above. When this is done, the sum of relative quantum yield as a function of the formal hydrogen ion concentration at each of the organic cosolvent mole fractions investigated was generally found to be within 5 % of 1, sometimes up though 0.1 M $[H^+]$. This observation provides support to the validity of this method. Further, normalization provided a

consistent means by which the effects of the individual solvents on the reprotonation rate constant could be compared.

Time-resolved Emission Spectroscopy

Emission decay measurements were carried out with the time-correlated single photon counting technique (147). A generalized schematic diagram of the instrumentation is shown in Figure 2.1, below. The important components are the start photomultiplier tube (PMT), the stop PMT, the flash lamp (L), the time-to-amplitude converter (TAC), the multichannel pulse height analyzer (MCPHA) and of course, the sample (S).

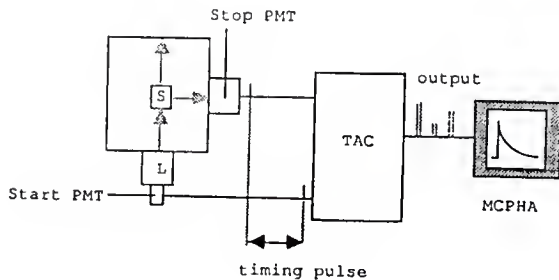


Figure 2.1. Time-correlated single photon counting instrument schematic.

The basic principles behind emission decay measurements are as follows. A pulse of light (L), simultaneously excites the sample (S) and initiates the start PMT which signals the TAC, whose function it is to charge a capacitor linearly with time. After some period of time, t , the sample may or may not emit a photon. If it does, the photon is detected by the stop PMT which in turn signals the TAC to stop charging. Thus, the voltage stored by the TAC is proportional to the time period between start and stop pulses. If no photon is detected after a predefined period of time, then the TAC resets itself and prepares for a new start pulse. However, if a signal is collected, the voltage is sent to the MCPHA, whose function is to digitalize and store the analog signal as a single count in a memory location or channel that corresponds to the pulse height. Thus, the pulse height is also proportional to the time that the photon is detected after initiation of the start pulse.

Quantitatively, the observed decay curve generated by the MCPHA is a histogram that describes the probability that a sample will emit a photon as a function of time following excitation. If the distortions from the instrument response time which arise because the excitation pulse is not infinitely narrow in width (a typical pulse is ~ 2 ns in width) are accounted for, the histogram collected on the MCPHA would correspond exactly to the excited state profile

of the emitting species. However, since the timescale of most emissive decays is generally in the nanosecond time regime, the observed emissive decay is a convoluted function of the time profile of the excitation pulse and the decay of the excited probe.

The observed decay function $R(t)$ can be expressed as a function of the time profile of the excitation pulse $L(t)$ and the impulse response of the sample as (147):

$$R(t) = \int_0^t L(t') F(t-t') dt' \quad (2.1)$$

where $F(t-t')$ is the response function of the probe and $L(t')$ is the time distribution of the lamp. The derivation for this expression has been given elsewhere (158,159).

To obtain the undistorted sample decay function, $F(t)$, from the experimentally determined functions $R(t)$ and $L(t)$, the method of iterative convolution is employed (145,147). This procedure convolutes an assumed decay law $F_c(t)$ of the form given by equation (1.40) and generates a calculated decay function $R_c(t)$ while minimizing the deviation between the experimentally observed decay, $R(t)$, and the calculated decay, $R_c(t)$. The goodness of fit is statistically determined from the reduced chi-square χ_r^2 :

$$\chi^2 = \sum_{i=1}^{n_1} W_i [R(t) - R_c(t)]^2 \quad (2.2)$$

$$\chi_r^2 = \frac{\chi^2}{n_2 - n_1 + 1 - p} \quad (2.3)$$

where χ^2 is the statistical chi-square, $W_i = 1/R(t)$ is a weighting factor for the i th data point, $n_1 + n_2$ are the starting and ending channels, respectively, of the MCPHA that are being fit and p are the number of variables in the fitting function (160).

Fits for convolution are considered to be good if χ_r^2 are ≤ 1.2 . A poor fit is distinguished by a $\chi_r^2 \geq 2$ (145,147,160).

Aqueous acid-base titrations

The probes examined had final concentrations of 10^{-5} M. They were prepared in water and known concentrations of H_2SO_4 and NaOH as noted in the steady-state section above. The emission wavelength for the neutral species of all probes examined was 350 nm. The emission wavelengths for the anionic species of the hydroxybiphenyls and cationic species of 6-methoxyquinoline were 418 nm and 450 nm, respectively.

Titration, performed at room temperature, were conducted as follows:

- 1) Two milliliters of the aqueous probe solution was pipetted into a 4 ml cuvette and placed into a photon counter equipped with a multi-channel analyzer. At least

5000 counts were collected in the channel of maximum intensity at each emission wavelength examined, provided a signal could be measured.

2) Two milliliters of a scatter solution (see below) was pipetted into a 4 ml cuvette and placed into the photon counter with the emission filter removed. The amount of photons collected in this step were the same as that obtained in step 1. The scatter solution was prepared by diluting a small amount of Kodak white reflectance coating in a solution that was identical to, but not containing the sample.

3) The counts obtained in steps 1 and 2 were subsequently downloaded to a computer fitted with a convolution program by PRA which allowed for the calculation of the emissive decay (i.e., lifetime).

4) The above solution was then titrated with either an acidic or basic probe solution and lifetime measurements were obtained after each increment of titrant was added, provided counts could be obtained. Calculation of the ionic strength at each point in the titration curve was carried out using the formal concentration of either acid or base.

Aqueous-organic solvent titrations

Working solutions of 2-hydroxybiphenyl were prepared as noted in the steady state section above. The lifetime (τ) of the neutral species in water was obtained as described

above. The aqueous solution was subsequently titrated with the organic probe solution. Lifetime were collected after each increment of titrant was added. The titration was carried out throughout the entire organic cosolvent mole fraction range.

Aqueous-organic acid-base titrations

Working solutions of 2-hydroxybiphenyl in 0.01 N NaOH at various mole fractions of organic cosolvent were prepared as noted in the steady-state methods section above, such that lifetime could be obtained for the 2-hydroxybiphenylate anion (τ_o).

CHAPTER 3 RESULTS AND DISCUSSION

Excited State Behavior in Aqueous Media

6-methoxyquinoline

Steady-state fluorescence

The fluorescence spectra of the isolated species of 6-methoxyquinoline (6MQ) is shown in figure 3.1. Notice that the emission maximum of the neutral or basic form of 6MQ, which is isolated in alkaline solutions when $\text{pH} \geq 13$ is 368 nm and that the emission maximum of the charged or acidic species of 6MQ, which is isolated in acidic media when $\text{pH} \leq 2$ is 440 nm. When the relative fluorescence intensity (RFI) of 6MQ is plotted as a function of pH, as depicted in figure 3.2, one finds that emission from the neutral species is significantly reduced in the alkaline range. It has been suggested by Schulman et al. (152), that the observed decrease probably results from quenching by hydroxide ion. However, this behavior has never been quantified.

In steady state investigations of excited state proton transfer, the RFI of each species must be converted to their respective relative quantum yield (RQY) before the kinetic

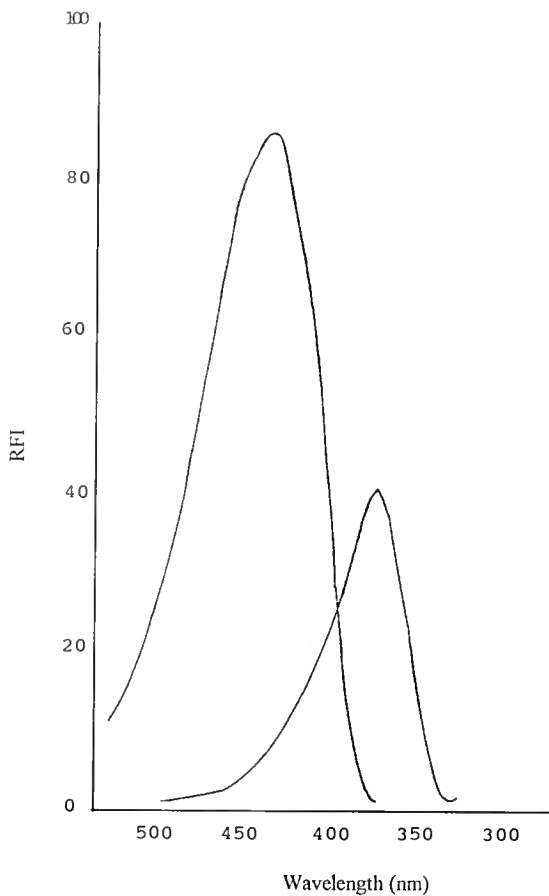


Figure 3.1. The fluorescence spectra of the acidic (A) and basic (B) species of 6-methoxyquinoline. The excitation wavelength was 289 nm.

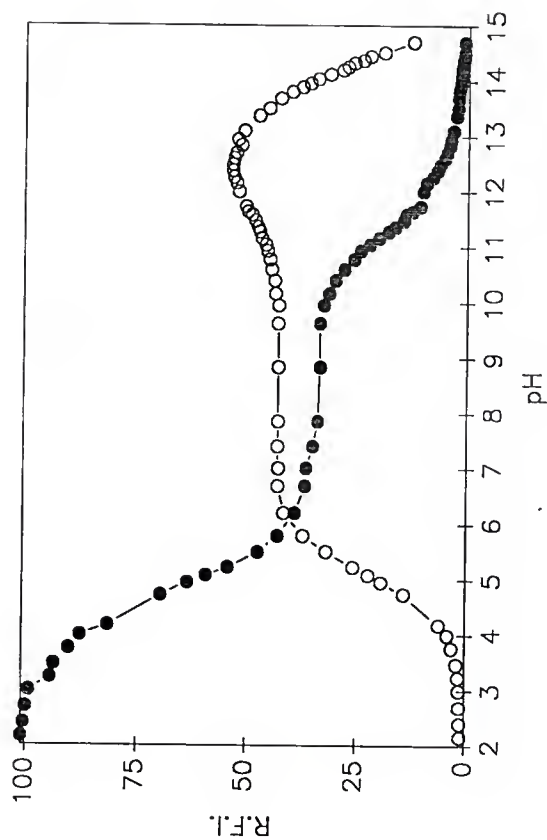


Figure 3.2. The RFI of the acidic (●) and basic (○) species of 6-methoxyquinoline as a function of pH.

rate constants can be determined. The procedure used for this conversion is generally straightforward and can be found in the methods section of Chapter 2, p.67. However, when the true fluorescence of an emitting species cannot be directly obtained, e.g., when quenching occurs, the process of generating accurate and reliable RQY is often quite difficult.

In an investigation of 6MQ by Schulman et al. (152), the RQY of the neutral or quenched species of 6MQ (ϕ/ϕ_0) were estimated by plotting $1 - (\phi'/\phi'_0)$ against pH, where ϕ'/ϕ'_0 is the RQY of fluorescence of the 6MQ cation, with the assumption that the RQY of the cationic species were true values. Unfortunately, this was not the case. As a result, the rate constants generated were in error. A more effective means of estimating the RQY of neutral 6MQ was achieved by extrapolating the RFI of the neutral species as a function of pH in the range where quenching plays a major role as depicted in figure 3.3. The RFI maxima obtained with this procedure was then used to generate reliable RQY. The fact that the sum of RQY for 6MQ approaches unity ($\phi/\phi_0 + \phi'/\phi'_0 = 1$) in the pH range where quenching does not play a significant role as shown in figure 3.4, provides support for this technique.

The quenching of fluorescence with a consequent decrease in the observed fluorescence lifetime of an excited

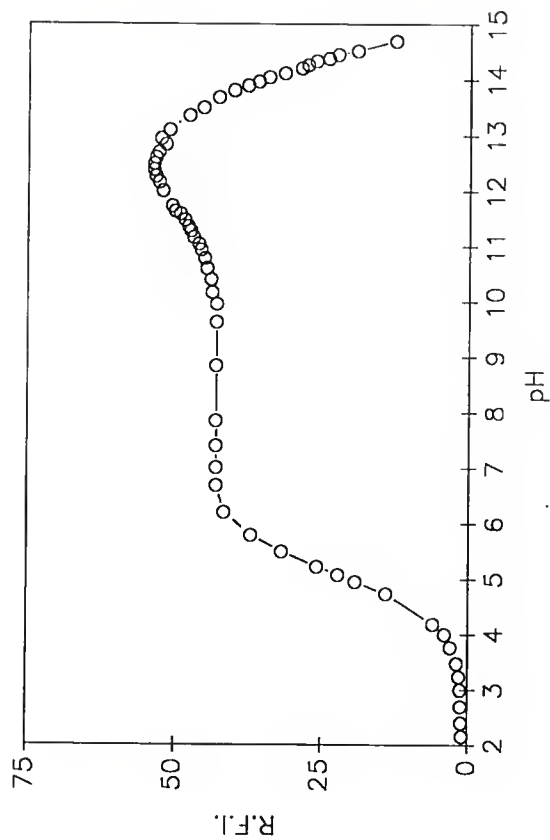


Figure 3.3. Extrapolation of the RFI in the alkaline range to estimate the RFI maxima of the neutral species of 6-methoxyquinoline

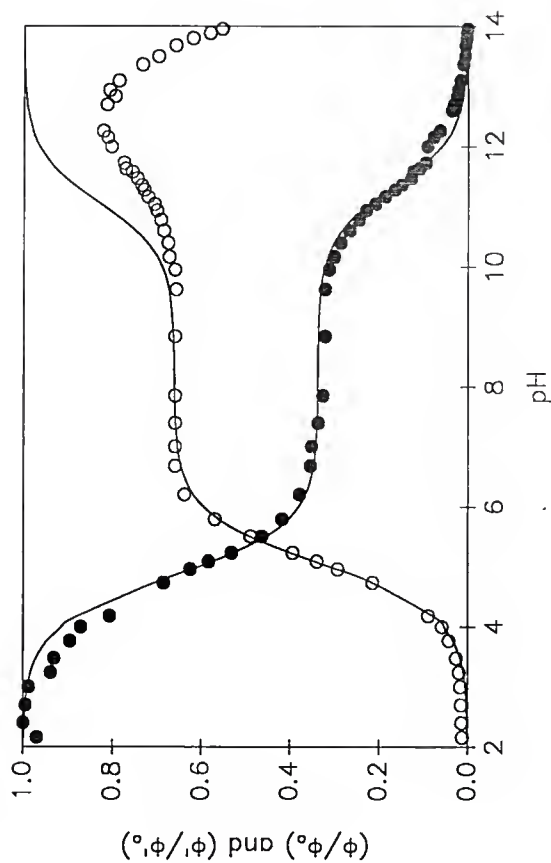
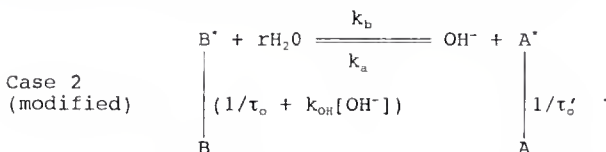


Figure 3.4. The observed RQY of acidic (●) and basic (○) 6-methoxyquinoline as a function of pH. The theoretical RQY (—) for the acidic and basic species were generated with equations (1.21) and (3.2), respectively.

species is a common photochemical event which must be accounted for in the kinetic expressions (161). One of the major prerequisites of traditional steady-state kinetic methods is that there should be no pH dependent quenching (141). However, in cases where the kinetic rate constants are much larger than the quenching rate constant, steady-state methods can still be used to provide reasonable estimates of the individual kinetic rate constants. For example, it was assumed in the development of Case 2 that quenching does not occur. However, since quenching has been shown to influence the emission of the neutral species, its behavior must be accounted for. This is achieved through a slight modification of Case 2 as shown below.



The only noticeable difference is the inclusion of $k_{OH}[OH^-]$ in the deactivation pathway of B^* , where k_{OH} is the hydroxide ion quenching rate constant and $[OH^-]$ is the molar concentration of hydroxide ion.

To determine the rate constants of protonation and deprotonation in Case 3, the following equations are integrated:

$$-\int_{t_1}^0 d[B^*] = \int_0^\infty \left(\frac{1}{\tau_0} + k_b + k_{OH}[OH^-] \right) [B^*] dt - \int_0^\infty k_a[OH^-][A^*] dt \quad (3.1)$$

$$-\int_{t_1}^\infty d[A^*] = \int_0^\infty \left(\frac{1}{\tau_0} + k_a[OH^-] \right) dt - \int_0^\infty k_b[B^*] dt \quad (1.20)$$

and subsequently rearranged to obtain the following expressions of RQY for excited base (B^*) and excited acid (A^*), respectively.

$$\frac{\phi}{\phi_0} = \frac{\alpha_B + k_a\tau'_0[OH^-]}{1 + k_b\tau_0 + (k_{OH}\tau_0 + k_a\tau'_0)[OH^-]} \quad (3.2)$$

$$\frac{\phi'}{\phi'_0} = \frac{\alpha_A + k_b\tau_0}{1 + k_b\tau_0 + k_a\tau'_0[OH^-]} \quad (1.22)$$

When equations (3.2) and (1.22) are combined, the following complex expression is obtained.

$$\frac{\phi/\phi_0}{\phi'/\phi'_0} = \frac{\alpha_B + k_a\tau'_0[OH^-]}{1 + k_b\tau_0 + (k_{OH}\tau_0 + k_a\tau'_0)[OH^-]} \cdot \frac{1 + k_b\tau_0 + k_a\tau'_0[OH^-]}{\alpha_A + k_b\tau_0} \quad (3.3)$$

Unlike equation (1.23) in case 2, the complex expression found above does not readily allow for the direct

determination of $k_a\tau'_0$ and $k_b\tau_0$ unless $k_{OH}\tau_0 \ll k_a\tau'_0$.

Therefore, it is essential that the effects of hydroxide ion quenching be examined.

When $\text{pH} > \text{pK}_a'$ by approximately 2 units, the following Stern-Volmer relationship, derived elsewhere (162), accurately describes the effect that the hydroxide ion has on the electronically-excited singlet state of neutral 6MQ:

$$\frac{\phi_0}{\phi} = 1 + (k_{OH}\tau_0)F[OH^-] \quad (3.4)$$

where ϕ_0 and τ_0 are the fluorescence efficiency and decay time of neutral 6MQ, B^* , in the absence of quencher and proton transfer, ϕ is the fluorescence efficiency in the presence of quencher, F is the Brønsted kinetic activity factor which corrects for ionic effect, and k_{OH} is the quenching rate constant of B^* by hydroxide ion..

From this expression, the rate constant of quenching is obtained by dividing the slope ($k_{OH}\tau_0$), of a plot of ϕ_0/ϕ versus $F[OH^-]$, as depicted in figure 3.5, by τ_0 . However, since collisional quenching of neutral 6MQ by hydroxide ion is a rate process which depopulates the excited state, τ_0 could not be measured directly. In an effort to acquire a reasonable and accurate estimate of τ_0 , two independent approaches were employed. In the first method, the observed lifetime (τ) and RQY (ϕ/ϕ_0) of neutral 6MQ were measured at

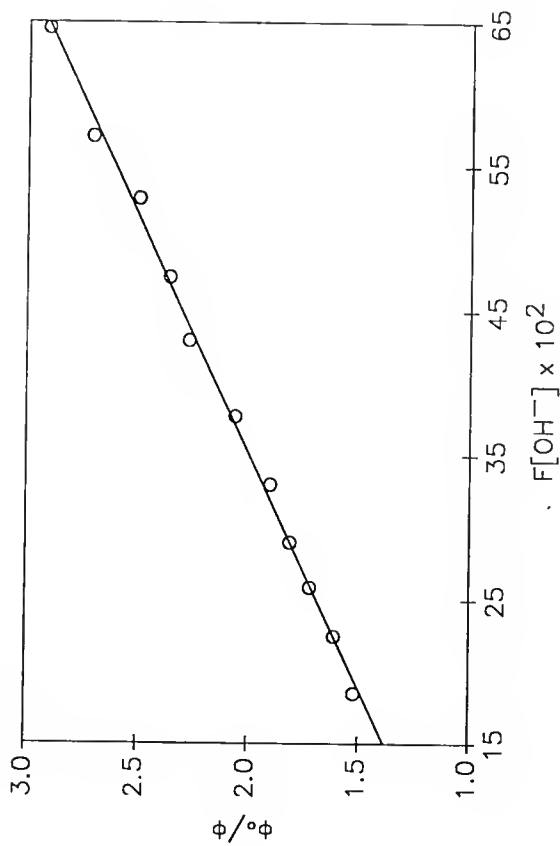


Figure 3.5. Variation of ϕ_0/ϕ for the neutral species of 6-methoxyquinoline as a function of $F[OH^-]$.

pH 7.0 to avoid the quenching effects of hydroxide. The data obtained was subsequently placed into the following relationship (141); $\tau = (\phi/\phi_0)\tau_0$, where it was found that $\tau_0 = 2.58 \pm 0.04 \times 10^{-9}$ s. In the second method, which is described at length in the time-resolved section below, $\tau_0 = 2.56 \pm 0.04 \times 10^{-9}$ s. These independently determined lifetime are not significantly different from each other (t-test; $P < 0.05$). Thus, k_{OH} which was computed from the slope of 3.5 is $1.18 \pm 0.04 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This value is consistent with the diffusional rates of small ions in solution (162). Yet, when the quenching rate constant is used in equation 3.2, one finds, as depicted in figure 3.4, that the theoretical RQY do not coincide with the observed RQY. The rationale for this discrepancy is not entirely clear. Since the system investigated was not degassed, one possible explanation is that molecular oxygen contributes to the observed behavior since molecular oxygen has been shown to quench the fluorescence of most compounds (162). Another possible explanation is that the observable increase in the turbidity of the solution, which occurred as the concentration of sodium hydroxide was increased, produced some apparent quenching. Perhaps all three processes play a role in the observed behavior. However, at the present time one can only speculate. The results of the hydroxide ion quenching experiment are presented in Table 3.1, below.

Table 3.1. The Stern-Volmer constant, relative quantum yield lifetime estimates and hydroxide ion quenching rate constant of neutral 6-methoxyquinoline.

pH	Slope ^a	τ^b	ϕ/ϕ_o^c	τ_o^d	k_{OH}^e
7.0	3.1 ± 0.1	1.72 ± 0.01	0.67 ± 0.01	2.58 ± 0.04	1.18 ± 0.03
				2.56 ± 0.04	1.19 ± 0.04

- a) The Stern-Volmer constant, $k_{OH}\tau_o$; units are M^{-1} . In all cases, ($\pm$) indicates standard error.
- b) Observed lifetime; units are ns. Previously reported values are 1.9 ns at pH 6.0 (43), 10.7 ns at pH 12.2 (152), 1.8 ns at pH 6.5 and 2.2 ns at pH 12.0 (153).
- c) Relative quantum yield.
- d) Estimated lifetime in the absence of quenching and proton transfer estimated from $(\phi/\phi_o)\tau_o$ at pH 7.0 and from biexponential decay data as defined by equation (55). Units are ns. A value of 4 ns has been reported (43).
- e) Hydroxide ion quenching rate constant; units are $\times 10^9 M^{-1} s^{-1}$.

It was noted above, that the individual prototropic rate constants of 6MQ could not be directly obtained, unless $k_a\tau_o' \gg k_{OH}\tau_o$. From Table 3.1, one finds that $k_{OH}\tau_o = 3.1$. Now, since the second order deprotonation rate constant of

6MQ by hydroxide ion is diffusion controlled ($k_a \approx 3 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$) (43,152) and the lifetime of the conjugate acid in the absence of proton transfer has been reported to be 23.7 ns (152), then $k_a\tau_o'$ is about 700 and $k_a\tau_o' \gg k_{OH}\tau_c$. Under these conditions equation (3.3) can be reduced to equation (1.23) and then further simplified to equations (1.24) and (1.25), as noted in the development of Case 2, to allow for the direct determination of the kinetic rate constants. Thus, even though hydroxide ion quenching plays a role in the observed RQY behavior of the neutral species, its effects become insignificant when appropriate corrections are made.

In addition to providing a means by which the kinetic rate constants of a particular system can be obtained, the RQY expressions provide a means by which the pH dependent emission of 6MQ can be understood. From equations (1.21) and (3.2) and figure 3.4, one finds that the pH dependent behavior associated with the RQY originates predominantly from the ground state characteristics of 6MQ in acidic solutions. For example, at $\text{pH} \leq 3$, only the RQY of the conjugate acid is observed. This results from the fact that this is the only species absorbing energy ($\alpha_B = 0$). However, in the pH range 3-7 ($\text{pK}_a = 5.13$; $\alpha_A = \alpha_B$), one finds that the RQY of this species decreases to 0.33, while the RQY of the neutral species appears and rises to 0.67. In this pH range

both species absorb and emit energy. However, when the pH exceeds the ground state pKa by about 2 units, the neutral species of 6MQ becomes the sole absorber ($\alpha_a = 0$). One would think that emission from the acidic species would fall to zero. Instead, one finds that the RQY of both species remain constant. This phenomenon results from the protonation, in the loHxst excited singlet state, of the pyridine nitrogen of the directly excited neutral 6MQ (142). One finds in this region of acidity or alkalinity (pH 6.7-10.7) that the RQY are related to the kinetics of protonation in the excited state by equation (1.25). Thus, from figure 3.4, $k_b\tau_o = 0.50 \pm 0.02$. Further, since $\tau_o = 2.57 \pm 0.04$ ns, k_b is $1.98 \pm 0.03 \times 10^8$ s⁻¹. As the pH exceeds 10, the RQY of the conjugate acid finally decreases and eventually disappears, while the RQY of the neutral species rises to a maximum of 0.84 at pH 12.4 before falling sharply with further increases in pH. The excited state reaction becomes dependent on pH and reversible. Further, one finds that the RQY are related to the kinetics of the system by equation (1.24). Thus, from a plot of $(\phi/\phi_o)/(\phi'/\phi'_o)$ versus $F[OH^-]$ as depicted in figure 3.6, where all terms have previously been defined, the constant, $k_b\tau_o$, obtained from the intercept was found to be 0.48 ± 0.08 , which is not significantly different from the value obtained with equation (1.25) (t-test; $P < 0.05$). In turn, the constant, $k_a\tau'_o$; determined

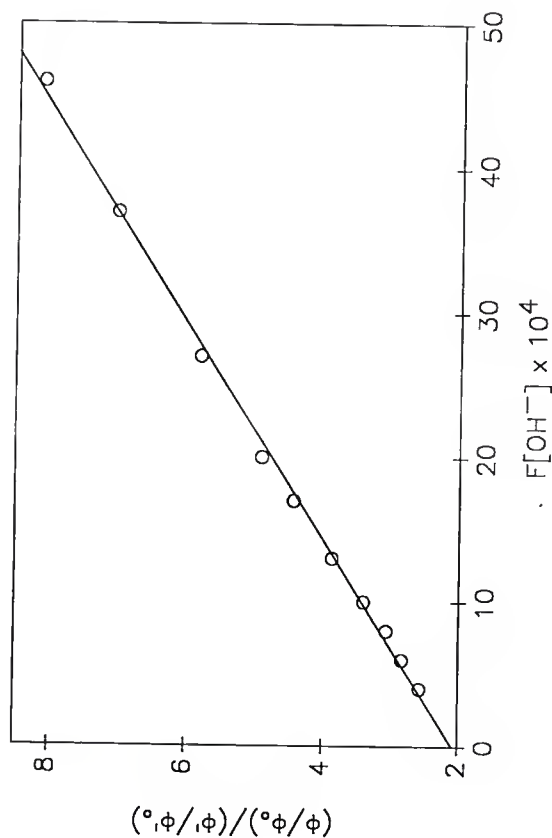


Figure 3.6. The variation of the relative quantum yield ratio $(\phi/\phi_0)/(\phi'/\phi'_0)$ of 6-methoxyquinoline as a function of $F[\text{OH}^-]$.

from the product of the slope of figure 3.6 and $k_b\tau_o$, which is obtained from either the intercept or equation (1.25) is $655 \pm 38 \text{ M}^{-1}$. Now, since τ'_o (the lifetime of the conjugate acid in the absence of proton transfer) was found to be $23.3 \pm 0.1 \text{ ns}$, $k_a = 2.8 \pm 0.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.

Finally, from the individual prototropic rate constants of 6MQ in the lowest excited singlet state, one can obtain the hydrolysis constant, $K_b^* = (k_b/k_a) = 6.9 \pm 0.4 \times 10^{-3}$. Therefore, the pK_a^* is 11.84 ± 0.03 . The individual rate constants and the ground and excited state dissociation constants have been compiled in Table 3.2 below.

Time-resolved fluorescence

At any given wavenumber the time-resolved decay of fluorescence for 6MQ is given by equation (1.40). From this expression, one would expect the observed emission decay profile to be biexponential. However, when time-resolved measurements were carried out on the conjugate acid (A^*) at 450 nm, the decay profile was found to be monoexponential, constant and independent of pH up through pH 10. Beyond this point, reminiscent of the RQY of this species, the lifetime decreased and eventually went to zero. A typical emission profile for A^* at pH 3.0 is shown in figure 3.7. The decay profile of the basic species, B^* , which was monitored at 350 nm is much more complicated. In the pH range 5-10, its emission decay profile is also monoexponential as depicted

Table 3.2. Experimentally determined constants, lifetime and calculated rate hydrolysis and dissociation constants of 6-methoxyquinoline in the lowest excited singlet (S_1) state.

$k_B \tau_o^a$	τ_o^b	k_B^c	$k_B \tau_o'^d$	$\tau_o'^e$	k_a^f	K_B^g	pK_a^h
<u>Steady-State Methods</u>							
0.48 ± 0.08	2.58 ± 0.04	1.86 ± 0.31	627 ± 105	23.3 ± 0.1	2.7 ± 0.5	6.9 ± 1.3	11.84 ± 0.09
0.50 ± 0.02		1.94 ± 0.03	655 ± 38		2.8 ± 0.2	6.9 ± 0.4	11.84 ± 0.03
<u>Time-Resolved Methods</u>							
		1.93 ± 0.03			3.0 ± 0.2	6.4 ± 0.4	11.81 ± 0.03

- Experimental constant obtained from equations (1.24) and (1.25), respectively. In all cases ($\pm$) indicates standard error.
- Lifetime of B' in the absence of proton transfer. Units are ns. A value of 4 ns has been reported (43).
- The protonation constant. In the steady-state section obtained from a/b. In the time-resolved section obtained from the difference of lifetime method. Units are $\times 10^8 \text{ s}^{-1}$. Previously reported values are $0.64 \times 10^8 \text{ s}^{-1}$ (152), $1.2 \times 10^8 \text{ s}^{-1}$ (153) and $2.0 \times 10^8 \text{ s}^{-1}$ (43).

Table 2--continued

- d) Experimental constant obtained from the product of the slope of figure 4 and $k_{\text{B}}\tau_0$. Units are M^{-1} .
- e) Lifetime of A' in the absence of proton transfer. Units are ns. A value of 23.7 ns has been reported (152).
- f) The deprotonation constant. In the steady-state section obtained from d/e. In the time-resolved section obtained from equation (52). Previously reported values are $2.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (152) and $3.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (43).
- g) The hydrolysis constant.
- h) The excited state pK_a . Previously reported values are 11.40 (152) and 11.80 (43).

in figure 3.7. However, in the pH range 11.48-12.40 a biexponential decay profile as depicted in figure 3.8 is found. The biexponential decay times of neutral 6-methoxyquinoline as a function of hydroxide ion concentration are listed with their respective chi-squared values in table 3.3, below.

Table 3.3. The biexponential decay times and chi-squared values of 6-methoxyquinoline as a function of hydroxide ion concentration. The excitation and emission wavelengths are 280 nm and 350 nm, respectively.

[OH ⁻]	τ_1 (ns)	τ_2 (ns)	χ^2
0.004	1.78 $\pm$ 0.02	10.28 $\pm$ 0.41	1.08
0.005	1.76 $\pm$ 0.02	8.61 $\pm$ 0.25	0.94
0.006	1.63 $\pm$ 0.02	7.44 $\pm$ 0.18	1.02
0.007	1.72 $\pm$ 0.02	7.22 $\pm$ 0.17	0.95
0.008	1.70 $\pm$ 0.03	6.35 $\pm$ 0.15	1.07
0.009	1.69 $\pm$ 0.03	6.20 $\pm$ 0.14	0.96
0.010	1.60 $\pm$ 0.03	5.53 $\pm$ 0.11	1.00
0.011	1.56 $\pm$ 0.03	5.11 $\pm$ 0.09	1.00
0.012	1.59 $\pm$ 0.05	4.82 $\pm$ 0.09	1.07
0.013	1.51 $\pm$ 0.04	4.69 $\pm$ 0.09	1.07
0.015	1.52 $\pm$ 0.05	4.33 $\pm$ 0.09	1.10

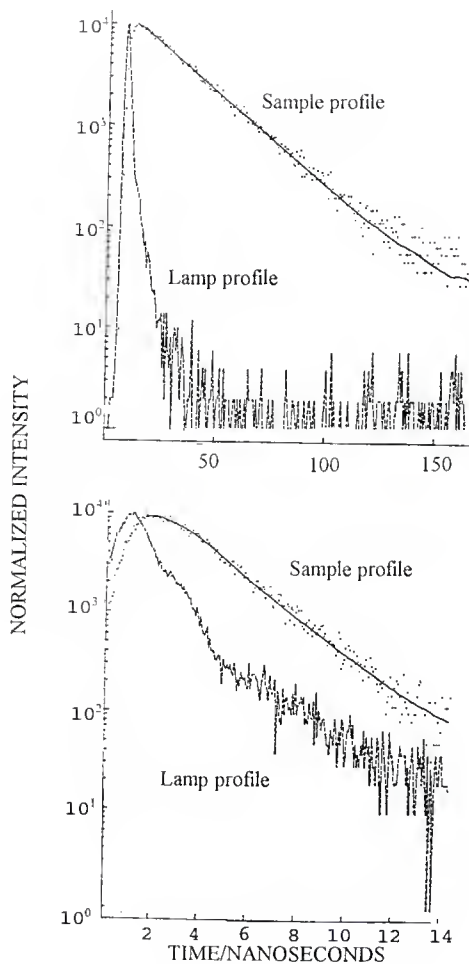


Figure 3.7. The monoexponential emission decay profiles of the acidic (upper) and basic (lower) species of 6-methoxyquinoline at pH 7.0.

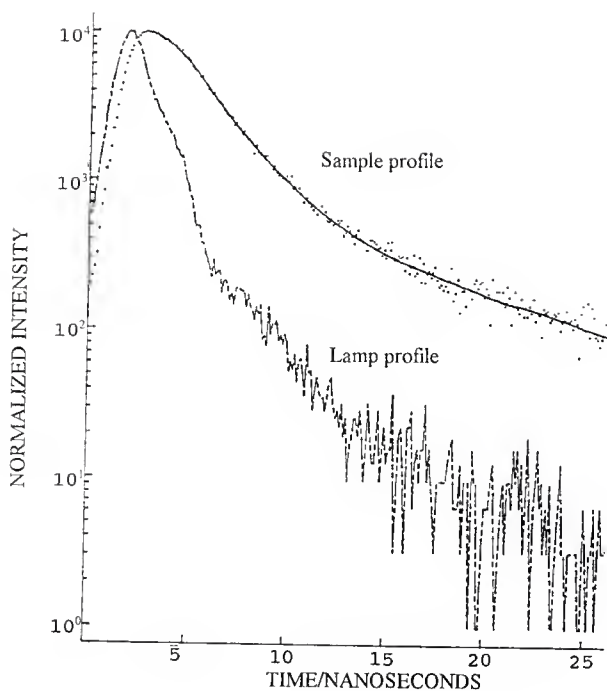


Figure 3.8. A biexponential emission decay profile of the basic species of 6-methoxyquinoline at pH 11.6.

The deviation from expected biexponential behavior might be ascribed to the fact that the observed decay times and pre-exponential factors of equation (1.40) are actually complex functions of the kinetic constants of the system. In addition, the pre-exponential factors are also dependent upon the spectral distribution of the species present. It would seem from the behavior depicted in figure 3.8, that the pre-exponential factor associated with each species at the wavelength in which the opposing species is monitored must be extremely low. In addition, one might speculate that the kinetic rate constants influence the observed decay profiles to a greater extent than do the pre-exponential factors because the biexponential behavior is only found when $\text{pH} = \text{pK}_a' \pm 0.5$.

From the monoexponential decay profile depicted in the upper portion of figure 3.7, the lifetime of the conjugate acid in the absence of proton transfer was found to be 23.3 ± 0.1 ns. Unfortunately, the lifetime of the basic species in the absence of proton transfer could not be obtained directly as noted earlier. To obtain a reasonable estimate of τ_o , indirect methods were used. The first method has already been described in the steady-state section above. Basically, it involved measuring both the RQY and lifetime of B' under conditions in which the observed emissive decay profile was found to be monoexponential. However, in the

second method, which has been used to estimate the lifetime of the neutral species of 2-naphthol when proton quenching prevented its direct measurement (148), the entire pH dependent-biexponential decay profile of B' is utilized. This technique has been adapted to the hydroxide quenching as follows. From equation (1.41), the following expressions can be obtained:

$$\gamma_1\gamma_2 = \gamma_A\gamma_B - k_b k_a [\text{OH}^-] \quad (3.5)$$

and

$$\gamma_1 + \gamma_2 = \gamma_A + \gamma_B = \gamma_A + \tau'_0 + k_a [\text{OH}^-] \quad (3.6)$$

Upon combination and elimination of $k_a[\text{OH}^-]$, the resulting expression is obtained:

$$\gamma_1 + \gamma_2 = \gamma_A \left(1 - \frac{\tau_0}{\tau'_0}\right) + \frac{1}{\tau'_0} + \tau_0 \gamma_1 \gamma_2 \quad (3.7)$$

A plot of $\gamma_1 + \gamma_2$ versus $\gamma_1\gamma_2$ should yield a straight line with a slope of τ_0 in the pH region where the reaction is reversible. From the slope depicted in figure 3.9, τ_0 was found to be 2.56 ± 0.06 ns. The fact that the lifetime obtained with both methods does not vary seems to validate this estimate. It also provides support for the original estimate of a fluorescence intensity maximum.

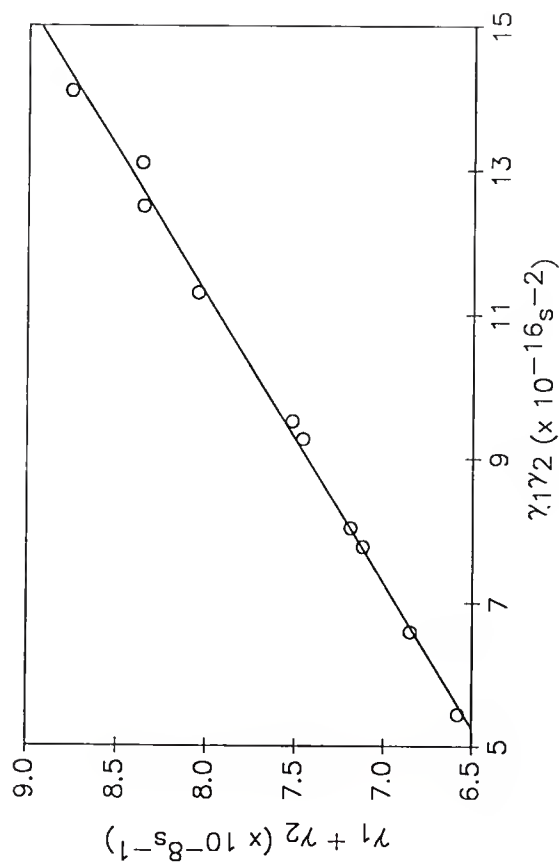


Figure 3.9. Variation of $\gamma_1 + \gamma_2$ as a function of $\gamma_1^{1/2}$

From the difference of lifetime method (see equations (1.47) and (1.48) in chapter 1) and the lifetimes of the neutral species in the absence of proton transfer and at pH 7.0, one can estimate the protonation rate constant without steady-state measurements. One find that $k_b = 1.93 \pm 0.03 \times 10^8 \text{ s}^{-1}$ which is not statistically different from the previous estimates (t-test; $P < 0.05$). In turn, the deprotonation rate constant of 6MQ can also be obtained directly with the time-resolved technique from the biexponential emission profiles collected over the pH range 11.5-12.2 and the sum of exponents as defined by the method of moments (150):

$$\gamma_1 + \gamma_2 = \gamma_A + \gamma_B = \frac{1}{\tau_0} + \frac{1}{\tau_0'} + k_b + (k_{OH} + k_a)F[OH^-] \quad (3.8)$$

where all constants have previously been defined. From the plot of $\gamma_1 + \gamma_2$ versus $F[OH^-]$ depicted in figure 3.10, the slope ($k_{OH} + k_a$) was found to be $3.1 \pm 0.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. Since $k_{OH} = 1.18 \pm 0.04 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, the deprotonation rate constant or k_a was $3.0 \pm 0.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. This value is identical to the estimate obtained at steady-state.

It is felt that the disparity found in the literature as to the excited state behavior of 6MQ in aqueous media probably stems from the technological limitations that

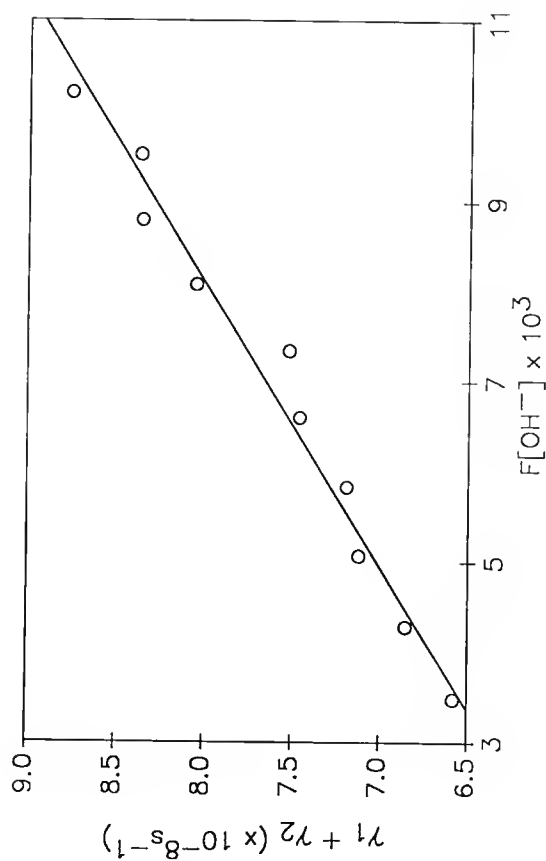


Figure 3.10. The variation in $\gamma_1 + \gamma_2$ as a function $F[OH^{-}]$.

existed when the original investigation of 6MQ was conducted. For example, in Schulman et al. (152) the emission decay profiles of 6MQ were measured on a nanosecond decay time fluorimeter which had a detection limit of about 2 nanoseconds. At that time it was deemed, that the lifetime of the neutral species in the absence of proton transfer or τ_0 was 10.8 ± 0.8 ns. However, using the time-correlated single photon counting technique in this study, τ_0 was found to be 2.56 ± 0.04 ns. Similar values have been obtained by others (43, 153). It is speculated that the machine used in the original investigation was not sensitive enough to detect the short-lived component of the biexponential profile. Instead, as seen from table 3.3, their lifetime estimate probably represents the much longer-lived component.

One can conclude that a combination of steady-state and time-resolved technique has substantiated Pines' et al. (43) claim that pK'_s is essentially 11.8 and not 11.4 as originally reported (152).

2-,3- and 4-hydroxybiphenyl

Steady-state fluorescence

Pertinent features of the absorption and fluorescence spectra of the hydroxybiphenyls are given in table 3.4. The pH dependencies of their fluorescence spectra in the form of

Table 3.4. Maxima of the longest wavelength absorption bands (${}^1L_b$ and ${}^1L_a$) and fluorescence bands of the neutral molecules (N) and anions (A) derived from the hydroxybiphenyls. Also presented are the fluorescence decay times of these species in the absence of proton transfer.

	ν^1L_a ($\text{cm}^{-1} \times 10^{-4}$)	ν^1L_b ($\text{cm}^{-1} \times 10^{-4}$)	ν_f ($\text{cm}^{-1} \times 10^{-4}$)	τ_f (ns)
<u>2-Hydroxybiphenyl</u>				
N	4.14	3.57	2.89	0.53 ± 0.04
A	4.00sh ^a	3.26	2.40	3.90 ± 0.01
<u>3-Hydroxybiphenyl</u>				
N	4.00	3.57sh ^b	2.94	1.50 ± 0.02
A	3.98sh ^a	3.33	2.34	2.62 ± 0.02
<u>4-Hydroxybiphenyl</u>				
N	3.91	C	2.97	1.24 ± 0.04
A	3.50	C	2.48	3.07 ± 0.01

a) Estimated inflection point of shoulder on the more intense ${}^1B$ band complex.

b) Estimated inflection point of shoulder on the more intense ${}^1L_a$ band.

c) The ${}^1L_b$ bands of the neutral species and anion, derived from 4-hydroxybiphenyl are completely eclipsed by the respective ${}^1L_a$ bands.

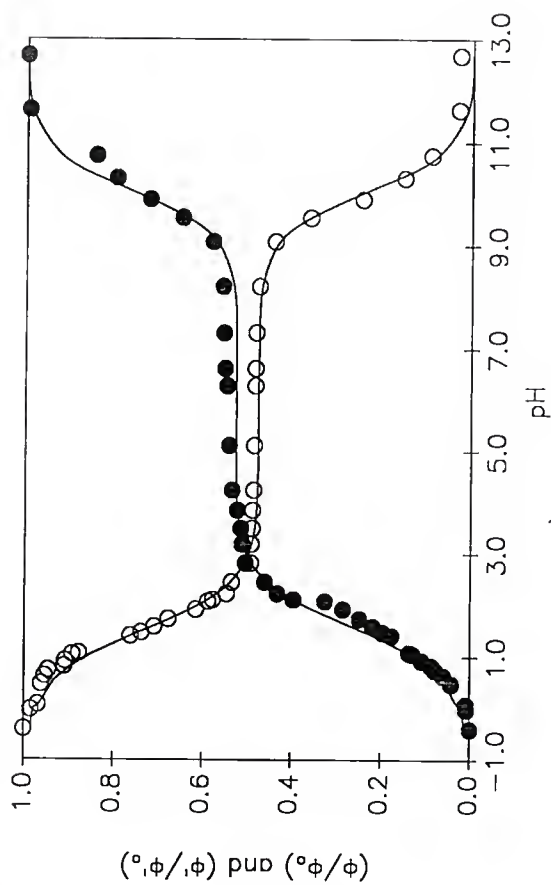


Figure 3.11. Variation in the relative quantum yield of 2-hydroxybiphenyl as a function of pH.

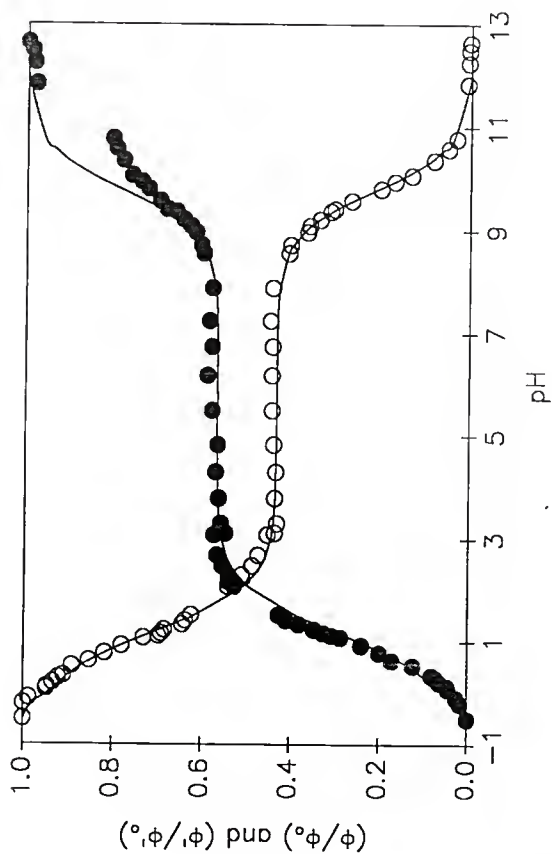


Figure 3.12. Variation in the relative quantum yield of 3-hydroxybiphenyl as a function of pH.

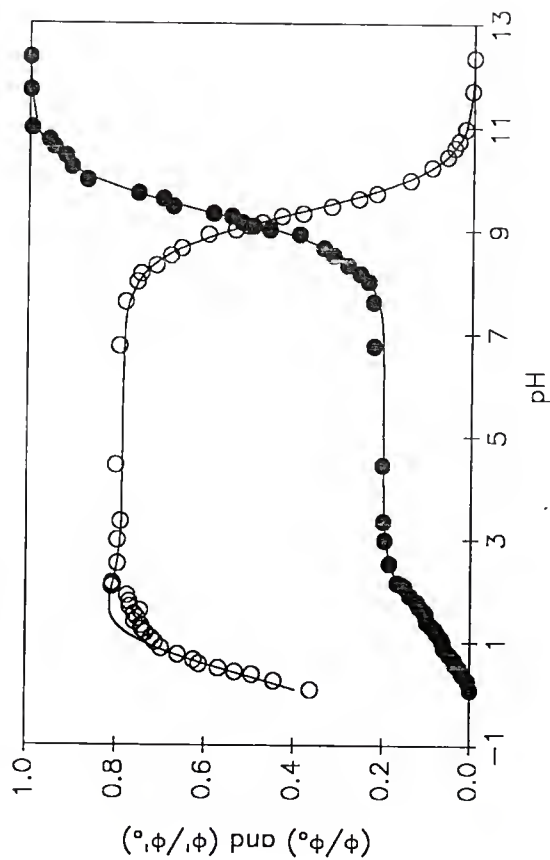


Figure 3.13. Variation in the relative quantum yield of 4-hydroxybiphenyl as a function of pH.

RQY are shown in figures 3.11, 3.12 and 3.13. Note that emission from both the neutral (N) and anionic (A) species remains constant in the pH range 2-8 even though excitation of the neutral species is virtually exclusive. This behavior results from the dissociation of the phenolic group in the lowest excited singlet state of the directly excited neutral molecules (141). It is consistent with the observations made by Bridges et al. (156), except that they reported that 4HBP did not exhibit prototropic dissociation in the lowest excited singlet state. This discrepancy can be explained by the fact that the primary analyte used by Bridges et al. contained 5 % (v/v) ethanol which has been shown to suppress the proton transfer process (vide infra). Further, in the pH range ≤ 3 , the excited state proton transfer kinetics of 4HBP are complicated by proton dependent quenching mechanisms which introduce a pH dependence to the RQY of the neutral species. Thus, in the absence of proton transfer, ϕ_0 must be calculated by an alternative method. Since proton dependent terms are negligible at neutral pH, the following relationship holds: $\phi_0 = \phi / (1 - \phi' / \phi'_0)$. This allows for a reasonable estimate of ϕ_0 to be made (161,164).

Using the estimate of ϕ_0 and steady-state techniques, it was found that the quenching of fluorescence for neutral 4HBP (N) is linearly related to both the hydrogen ion and bisulfate anion concentrations, based upon the following

Stern-Volmer relationships (162):

$$\frac{\phi_0}{\phi} = 1 + (k_H \tau_0) F[H^+] \quad (3.9)$$

$$\frac{\phi_0}{\phi} = 1 + (k_{HSO_4} \tau_0) F[HSO_4^-] \quad (3.10)$$

where ϕ_0 and τ_0 are the RQY and lifetime of N in the absence of quenching and proton transfer, ϕ is the RQY of N in the presence of these processes, F is the Bronsted kinetic activity factor (which compensates for ionic effects) and k_H and $k_{HSO_4^-}$ are the rate constants of quenching of by H^+ and HSO_4^- , respectively. From the slopes of figures 3.14 and 3.15 and from an estimate of τ_0 obtained at pH 1 using the relationship $\tau = (\phi/\phi_0)\tau_0$, the individual quenching rate constants were both found to be $2.3 \pm 0.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This is consistent with the diffusion rates of small ions in solution. When these quenching constants are used in the following RQY expression for N, derived as per case 3, equation (3.11),

$$\frac{\phi}{\phi_0} = \frac{\alpha_A + k_B \tau_0' [H^+]}{1 + k_A \tau_0 + (k_Q \tau_0 + k_A \tau_0') [H^+]} \quad (3.11)$$

one finds that the RQY obtained match the experimental data

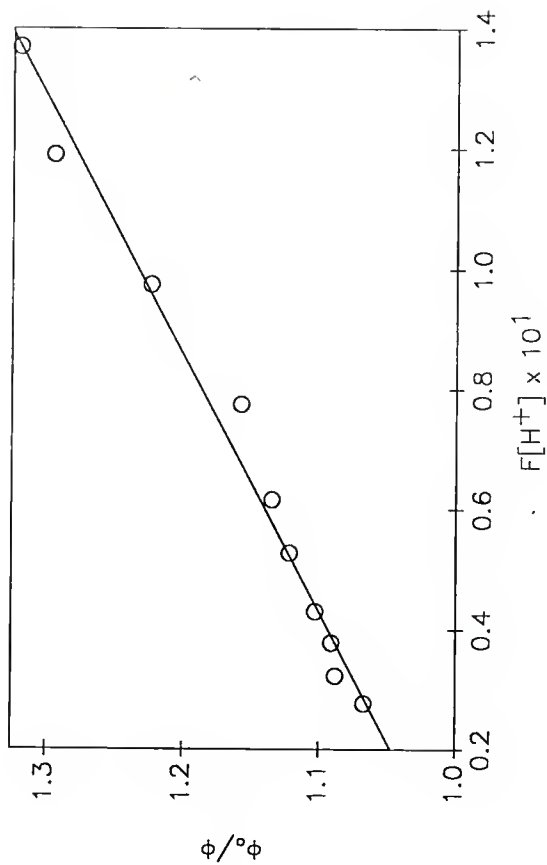


Figure 3.14. Variation of ϕ_0/ϕ of 4-hydroxybiphenyl as a function of $F[H^+]$.

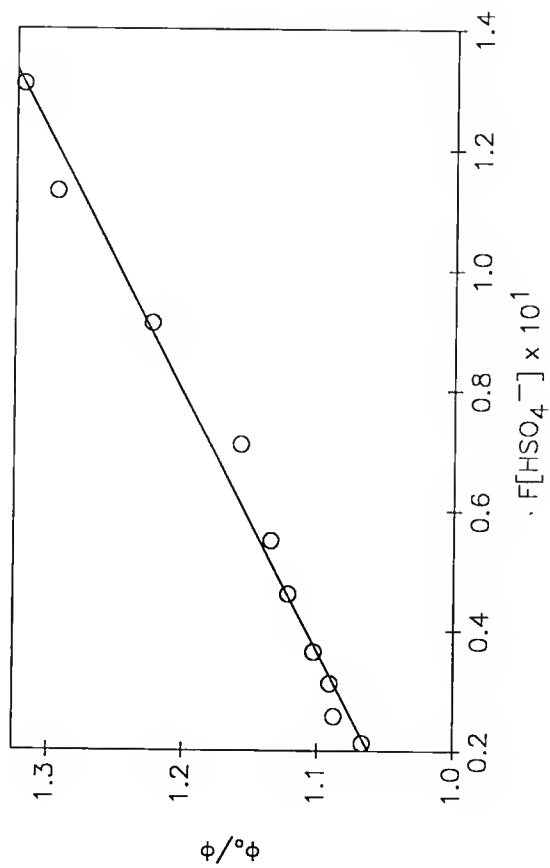


Figure 3.15. Variation of ϕ_0/ϕ of 4-hydroxybiphenyl as a function of $F[\text{HSO}_4^-]$.

very well. This is true provided the individual quenching rate constants are combined to give the observed quenching rate constant, $k_Q = (k_{H^+} + k_{HSO_4^-}) = 4.6 \pm 0.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.

An expression similar in form to equation (3.3) can be developed for 4HBP. However, since $k_b\tau_o' > k_o\tau_o$ by at least a factor of 10, the complex expression can be reduced to equation (1.15). This provides a means to calculate the individual rate constants of 4HBP and the remaining isomers, if the appropriate lifetime are available through graphical technique. Further, when the RQY of the hydroxybiphenyls are invariant to pH ($k_b\tau_o'F[H^+] \ll 1$ and $\alpha_b = 0$), equation (1.15) reduces to equation (1.17). Thus, one finds that the value of $k_a\tau_o$ for the 2-, 3- and 4-isomers can be calculated directly from figures (3.11), (3.12) and (3.13). The resulting values of $k_a\tau_o$ for each isomer can then be multiplied by the slopes of the plots of $(\phi/\phi_o)'/(\phi'/\phi_o')$ versus $F[H^+]$ depicted in figures (3.16), (3.17) and (3.18), to obtain estimates of $k_b\tau_o'$ (see equation (1.17)). Finally, with the appropriate lifetime values (see Table 3.4), one can generate the individual rate constants (k_a and k_b) of each isomer. are obtained. These values have been listed with the ground and excited state dissociation constants in Table 3.5.

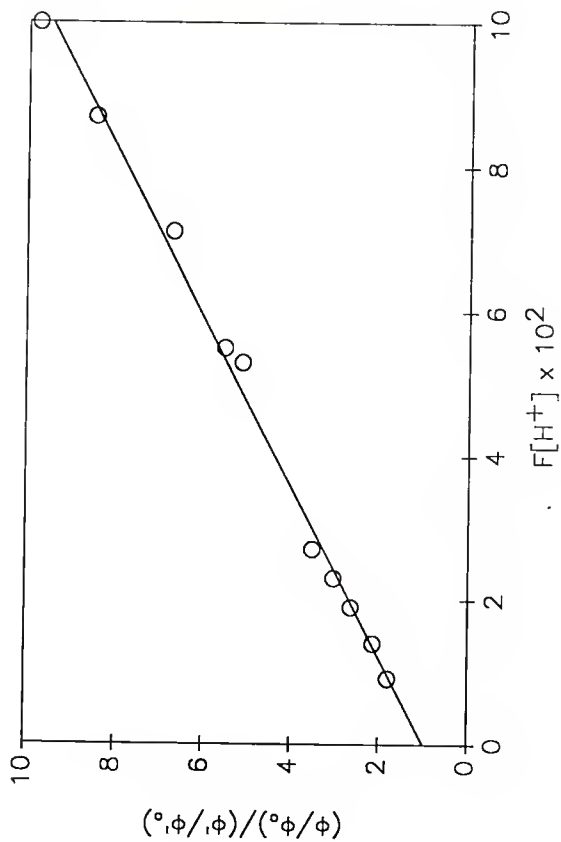


Figure 3.16. Variation of $(\phi/\phi_0)/(\phi'/\phi'_0)$ in 2-hydroxybiphenyl as a function of $F[H^+]$.

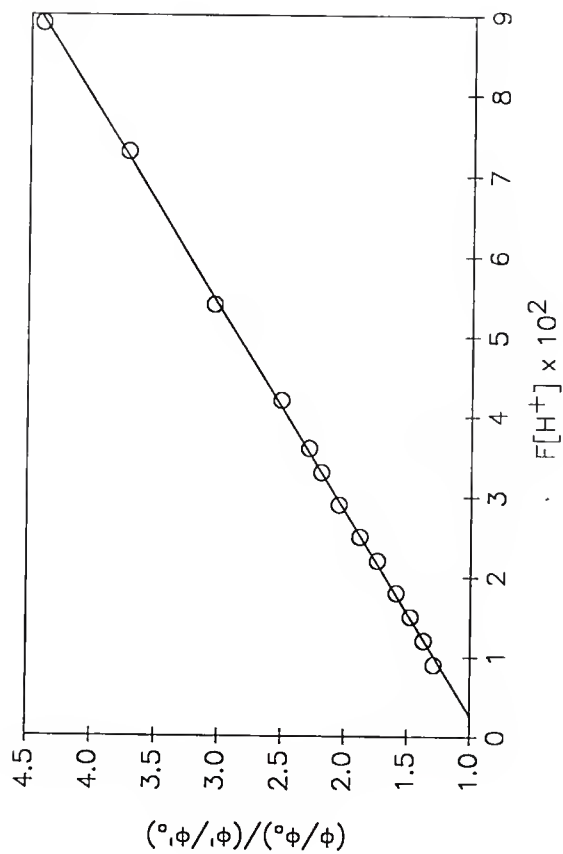


Figure 3.17. Variation of $(\phi/\phi_0)/(\phi'/\phi'_0)$ in 3-hydroxybiphenyl as a function of $F[H^+]$.

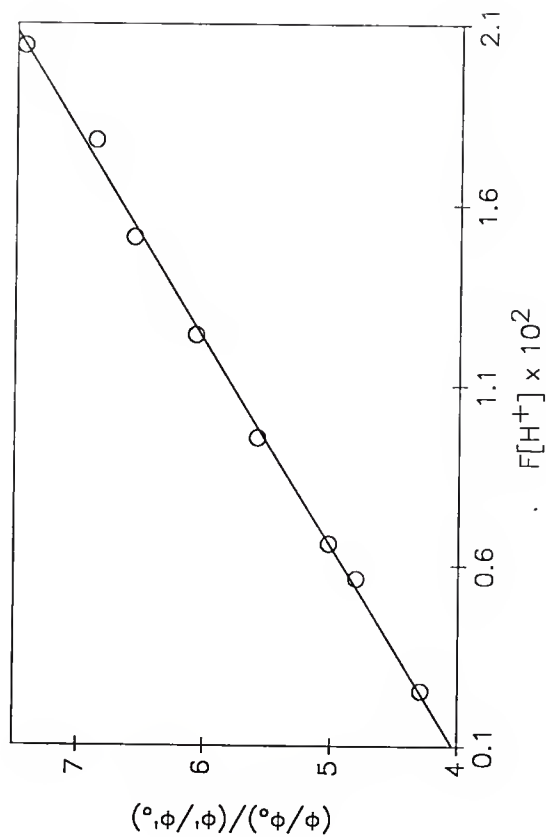


Figure 3.18. Variation of $(\phi/\phi_0)/(\phi'/\phi'_0)$ in 4-hydroxybiphenyl as a function of $F[H^+]$.

Table 3.5. Rate constants (k_a and k_b) for prototropic dissociation and reprotonation of the hydroxybiphenyls (HBPs) in the S_1 state and dissociation constants in ground (pK_g) and lowest excited singlet (pK_{S_1}) states.

	$pK_{S_0}^a$	k_a^b	k_a^c	k_b^d	k_b^e	$pK_{S_1}^f$	$pK_{S_1}^g$
<u>2-HBP</u>	10.09 ± 0.05	21 ± 2	16 ± 4	3.0 ± 0.1	2.3 ± 0.6	1.15 ± 0.04	1.16 ± 0.1
<u>3-HBP</u>	9.62 ± 0.01	8.5 ± 0.1	7.5 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.36 ± 0.03	1.36 ± 0.03
<u>4-HBP</u>	9.56 ± 0.03	2.2 ± 0.3	2.1 ± 0.5	1.5 ± 0.1	1.5 ± 0.4	1.83 ± 0.04	1.85 ± 0.08

- a) Ground state dissociation constant as determined spectrophotometrically in this lab. In all cases ($\pm$) indicates standard error.
- b) Dissociation rate constant calculated directly from equation (1.17).
- c) Dissociation rate constant obtained from the intercept of figures (3.16), (3.17) and (3.18).
- d) Reprotonation rate constant calculated from the product of b and the slopes of figures (3.16), (3.17) and (3.18).
- e) The reprotonation rate constant calculated from the product of c and the slopes of figures (3.16), (3.17) and (3.18).

Table 3.5--continued

- | | |
|----|---|
| f) | Excited state dissociation constant; $K_{b_1} = (k_a/k_b)$, where k_a and k_b are taken from b and d, above. |
| g) | Excited state dissociation constant; $K_{b_1} = (k_a/k_b)$, where k_a and k_b are taken from c and e, above. |

Time-resolved fluorescence

At any given wavenumber the time-resolved decay of fluorescence for the HBPs is given by equation (1.39). This suggests that the emission decay profiles should be biexponential. However, only monoexponential decay profiles were observed. Perhaps the rationale for this behavior stems from the fact that the HBPs do not emit a great deal of light, i.e., they have weak emission profiles. Thus, for the most part, lifetime determination was only possible at extremes of pH where the fluorescence intensity of these compounds is strongest and biexponential behavior is not usually seen. The lifetime estimates have been listed in Table 3.4.

From the rate constants for the dissociation and reprotonation shown in Table 3.5, it is seen that 2HBP exhibits more prototropic activity in the lowest excited singlet (S_1) state than do 3HBP and 4HBP, in that order. The reason for this behavior is not quite clear. However, if one recognizes that the π electron system of the HBPs consists of two distinct moieties connected by a formal single bond and that the inter-ring exciton interaction that exists between the individual subunits is probably quite large ($> 1000 \text{ cm}^{-1}$ for biphenyl (165), then the electronic states of HBPs can be described in relation to their individual subunits (166). Further, if the excitations from one subunit

to another are neglected, local excitations can be reduced to the interaction of the corresponding transition dipole moments (167). In this context, if the HBPs are considered to be substituted phenols, a sufficient qualitative description of the influence of substituent orientation on the acidity of the hydroxybiphenyls in the lowest excited singlet state might be obtained from the Platt free-electron model (168). This approach has already been used to explain the electronic spectra and excited state proton transfer of the cyanophenols (169). However, the HBPs, or for present purposes the phenylphenols, are a more complicated case as it is well known that biphenyl and its derivatives undergo large conformational changes upon excitation. In the ground state a $42 \pm 2^\circ$ dihedral angle exists between the two rings (154) while in the excited state the molecule becomes planar (155). Thus, unlike the cyanophenols where the rotation of the cyano group does not appreciably affect the energies of the lowest excited singlet states, the rotation of the phenyl group plays an important role in the photochemistry of the phenylphenols.

According to the Platt free-electron model (168), in disubstituted benzenes containing an electron withdrawing and an electron donating group, the longest wavelength $\pi \rightarrow \pi^*$ absorption band which is derived from the ${}^1L_b \leftarrow A$ transition of benzene is considerably red-shifted relative to the ${}^1L_b$

band of benzene in the case of o- and m- disubstitution. In p- disubstituted benzenes, however, the ${}^1L_b$ band is red-shifted relative to that of benzene to a much smaller degree. This is a consequence of the degree of charge transfer in the electronic transition to and from the benzene ring by the substituents. This in turn depends on how the substituents are aligned with the axis of polarization of the ${}^1L_b \leftarrow A$ transition of benzene. Each substituent lies at 30° in the case of the o- and m- disubstituted compounds and at 90° in the case of the p- disubstituted compounds with respect to the ${}^1L_b \leftarrow A$ transition moment axis of the substituted benzene. The second absorption bands (${}^1L_a$ bands) of the disubstituted benzenes are derived from the ${}^1L_a \leftarrow A$ transition of benzene. For this transition, substituents in the o- and m- disubstituted derivatives each make 60° angles with the axis of polarization of the ${}^1L_a \leftarrow A$ transition while in the p- disubstituted case the substituents are collinear with the ${}^1L_a \leftarrow A$ transition moment. Therefore, the ${}^1L_a$ band of the p- disubstituted molecules is very red-shifted relative to that of benzene while the ${}^1L_a$ bands of the o- and m- disubstituted derivatives are considerably less so.

Since the S_1 state of benzene is the ${}^1L_b$ state (170), one would assume that the S_1 state of the substituted phenols should also be the ${}^1L_b$ state. Thus, the change in

long wavelength absorption produced by ionizing the hydroxy group should be most pronounced in the o- and m-disubstituted phenols and less so in the p-disubstituted phenols. Presumably, the fluorescence of substituted phenols, which also originates from the ${}^1L_b$ state, should be most affected by ionization of the 2- and 3-disubstituted compounds. It therefore follows that the strongest effects on the prototropic dissociation rate and equilibrium constants in the S_1 state should be observed in the o- and m-derivatives and the weakest in the p-derivative. This has been observed in both the cyanophenols (169) and the phenylphenols where the o- and m-isomers demonstrate considerable excited state proton transfer in the lowest excited singlet state while the p-isomer does so to a lesser degree. It is the large angle (ca 90°) between the line joining the p-substituents and the ${}^1L_b \leftrightarrow A$ transition moment direction that accounts for the poor coupling of the phenyl group with the transition to the S_1 state, and hence, to the weak acidity of the p-isomer. This behavior is reminiscent of that of phenol itself in which there is no electron-withdrawing substituent to augment the acidity of the hydroxy group and there is no measurable proton transfer during the lifetime of S_1 . Because the substituent axis in the p-isomer is co-linear with the ${}^1L_a \leftrightarrow A$ transition moment direction, the ${}^1L_a$ state is lowered in energy much more than

the ${}^1L_b$ state accounting for the obscuring of the ${}^1L_b$ absorption band by the intense ${}^1L_a$ band. However, in the ${}^1L_a$ state the p-isomer is apparently not lowered enough for it to become the S_1 state. Otherwise the strong substituent effect would be reflected in a high rate constant for proton transfer in the S_1 state.

In the o- and m- isomers the electron withdrawing effect of the phenyl group is manifested in high rate constants for dissociation in S_1 and occurs because of the small angle (ca. 30°) between the ${}^1L_b \leftarrow {}^1A$ transition moment direction and the bond joining the two phenyl rings.

The rate constant for dissociation of the o- isomer is about 3 times larger than the m- isomer which is about 3 times larger than the p- isomer. This behavior is also seen in the cyanophenols, although to a lesser degree (169), which indicates that the phenyl group in the S_1 state is a very potent electron withdrawing group -- even stronger than the cyano group. Presumably, this is a result of the fact that, due to noncoplanarity of the two phenyl rings in the ground state and their consequent very weak interaction, the delocalization of the hydroxy group electrons into the expanded aromatic system subsequent to excitation and thermal relaxation in the S_1 state is greatly exaggerated by comparison with the degree of intramolecular charge transfer that occurs upon excitation in an aromatic system which is

planar in ground and excited states. In the latter case there is substantial delocalization over the entire aromatic system prior to electronic excitation so that the change in electron density at a hydroxy group is less pronounced than in, say, the phenylphenols. Further, there is also an extended conjugation system in the o- and p- isomers, which lowers the energy of their ^1L_b states. Thus, extended conjugation and substantial delocalization of the electrons into the ring system provides a means to explain the individual differences between the o-, m- and p- dissociation rate constants as well.

The rate constants for reprotonation of the dissociated phenylphenolates are typical of those observed for diffusion-controlled protonations (38,171). However, the fact that this rate constant is twice as large for the o- and m- isomers as for the p- isomer is somewhat puzzling. Usually, such differences for molecules of similar size and chemistry are due to steric factors which limit the accessibility of the site of protonation to the proton (3). However, it would seem that the o- isomer should have more steric restriction than the m- and p- isomers, and that this should yield the opposite order for the reprotonation rate constants. At present the reason for this apparent paradox remains unclear.

Excited State Behavior in Mixed Aqueous-Organic MediaCosolvent Effects on the Forward Rate Constant

The inhibitory effect of organic cosolvents on the protonation and dissociation rates of 6MQ and 2HBP, respectively, were examined with steady-state and time-resolved spectroscopy. The primary objective was to determine whether the transition state treatment that was developed with respect to the activity of water by Schulman and Kelly (74,75) would provide a basis through which the effects of organic cosolvent addition on the rate of proton transfer can be understood. However, before examining this novel steady-state approach to proton transfer, it was felt that the forward rate constant of 6MQ and 2HBP should be plotted against the mole fraction of organic cosolvent, since other physical chemical properties of mixed solvent systems such as the bulk dielectric constant, water activity and molecular volume, to name just a few, are frequently expressed in the same manner. In this way it may be easier to relate these parameter to the observed results.

6-methoxyquinoline

A series of aqueous solutions of 6MQ, with their pH fixed somewhere between 6.77 and 8.89, were titrated with ethanolic solutions of 6MQ. When the RQY ratios as defined by equation (1.25) were plotted as a function of the organic

cosolvent mole fraction (X_{ORG}), it was found that the rate constant of 6MQ protonation decreased biexponentially as depicted in figure 3.19. Further, when the individual data sets were fit to the following expression,

$$\frac{\Phi' / \Phi_0'}{\Phi / \Phi_0} = A_1 \cdot 10^{-(m_1 X_{ORG})} + A_2 \cdot 10^{-(m_2 X_{ORG})} \quad (3.12)$$

it was found that the intercepts (A_1 and A_2) and slopes (m_1 and m_2) obtained at each pH were essentially the same as noted in table 3.6. This indicates that as long as pH is maintained within the range where the RQY of each species is constant, i.e., the range in which only the forward reaction occurs, pH will not influence the effect that the organic cosolvents have on the forward rate constant. In addition, notice in table 3.6 that the terminal slope, m_2 , is essentially zero. In other words, proton transfer appears to be nonexistent once the mole fraction of ethanol exceeds 0.4. It therefore follows that equation (3.12) can be simplified to the following log-linear relationship.

$$\log\left(\frac{\Phi' / \Phi_0'}{\Phi / \Phi_0}\right) = \log(k\tau_0)_0 + mX_{ORG} \quad (1.9)$$

In equation (1.9) the intercept, $\log(k\tau_0)_0$ (a composite

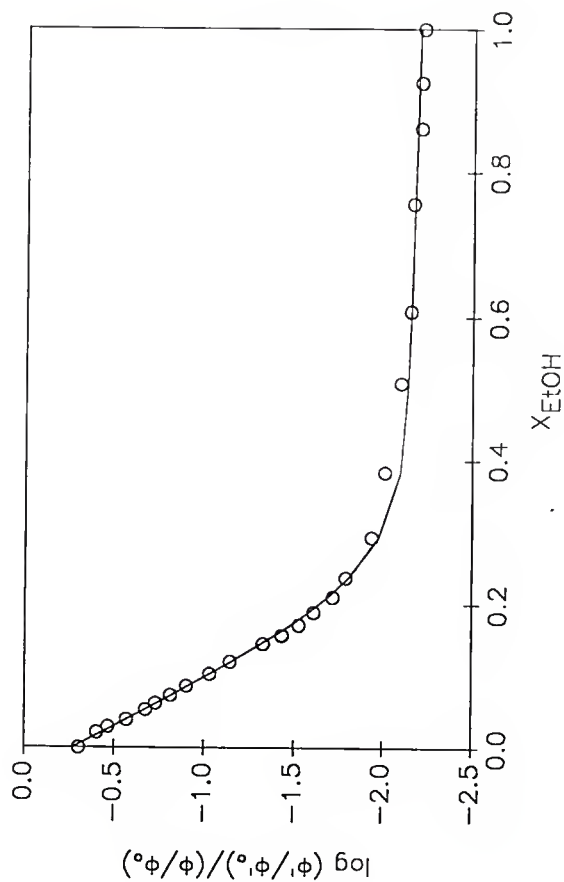


Figure 3.19. Plot of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ versus mole fraction of ethanol for 6-methoxyquinoline.

Table 3.6. Results of the biexponential fitting of the plots of $(\phi'/\phi'_0)/(\phi/\phi_0)$ versus mole fraction of ethanol for 6-methoxyquinoline.

pH	A_1	m_1	A_2	m_2
6.77	0.53 ± 0.01	-7.7 ± 0.2	0.008 ± 0.002	-0.1 ± 0.1
7.43	0.53 ± 0.01	-7.8 ± 0.2	0.008 ± 0.002	-0.1 ± 0.2
7.69	0.52 ± 0.01	-7.9 ± 0.2	0.009 ± 0.002	-0.2 ± 0.1
8.51	0.52 ± 0.01	-7.8 ± 0.2	0.009 ± 0.002	-0.2 ± 0.1
8.89	0.53 ± 0.01	-7.3 ± 0.2	0.009 ± 0.002	-0.1 ± 0.2

function of the forward rate constant and the lifetime of the neutral species of 6MQ) is equal to the ratio of quantum yields obtained in the pH independent portion of the aqueous titration curve as defined by equation (1.25). The slope m_1 is simply some energetic parameter related to the mixed solvent system.

A comparison of the inhibitory effects of ethanol on the protonation rate constant up through an organic cosolvent mole fraction of approximately 0.2 as a function of pH using equation (1.9) is given in table 3.7, below. Notice that the slope; $m = -7.4 \pm 0.2$, is identical to that obtained from the biexponential fitting; $m_1 = -7.7 \pm 0.2$. In addition, notice that these values coincide with results obtained by Kelly; $m = -7.9 \pm 0.1$ (151). Further, one finds that the intercept, $(k\tau_0)_0$, obtained in this current mixed

Table 3.7. Results of the regression analysis of the plots of $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ vs mole fraction of ethanol for 6-methoxyquinoline as a function of pH.

pH	Intercept	$(k\tau_0)_0$	Slope (m)	r^2
6.77	-0.28 ± 0.01	0.52	-7.3 ± 0.1	0.999
7.43	-0.27 ± 0.01	0.54	-7.4 ± 0.1	0.999
7.69	-0.28 ± 0.01	0.52	-7.5 ± 0.1	0.999
8.51	-0.27 ± 0.01	0.54	-7.5 ± 0.1	0.999
8.89	-0.26 ± 0.01	0.55	-7.1 ± 0.1	0.998
Mean Values	-0.27 ± 0.01	0.53 ± 0.01	-7.4 ± 0.2	

solvent investigation is essentially the same as that obtained in the aqueous investigation, i.e., $k\tau_0 = 0.50 \pm 0.02$. However, in Kelly's study a value of 1.22 was reported (151). The reason for this discrepancy comes from the fact that Kelly failed to characterize the excited state behavior of 6MQ in aqueous media. Had he done so, he would have realized that one must account for the quenching of neutral 6MQ by the hydroxide ion.

With Dr. Schulman's permission, the data produced by Kelly in aqueous ethanol was reevaluated and when appropriate corrections were made to the RQY, the slope and intercept values were found to be -7.3 ± 0.1 and 0.52, respectively; $r^2 = 0.999$. These values are identical to

those obtained in this study and as such, support the claim made above.

Steady-state titrations of 6MQ in aqueous methanol and aqueous acetonitrile were also carried out. The plots of $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ versus mole fraction were linear with slopes of -3.3 ± 0.1 and -10.3 ± 0.1 , respectively. These values coincided nicely with data generated by Kelly, once the appropriate RQY corrections were made. Therefore, it seemed logical to conclude that the initial titrations of Kelly reflect those obtained in this investigation. With Dr. Schulman's permission, all remaining raw data on 6MQ, with the exception of that taken in aqueous formamide, originates from the doctoral work of Kelly (151).

The slopes and the intercepts which were obtained from plots of $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ versus mole fraction for each of the organic cosolvent examined, which are depicted in figure 3.20, can be found in Table 3.8. Notice that the rate of protonation of 6MQ appears to be dependent upon the identity of the organic cosolvent. The rationale for this observed behavior will be discussed in greater detail after examining the inhibitory effect that the organic cosolvents have on the dissociation rate of 2HBP.

2-hydroxybiphenyl

When the RQY ratios of 2HBP were plotted against the organic cosolvent mole fraction, as depicted in figures 3.21

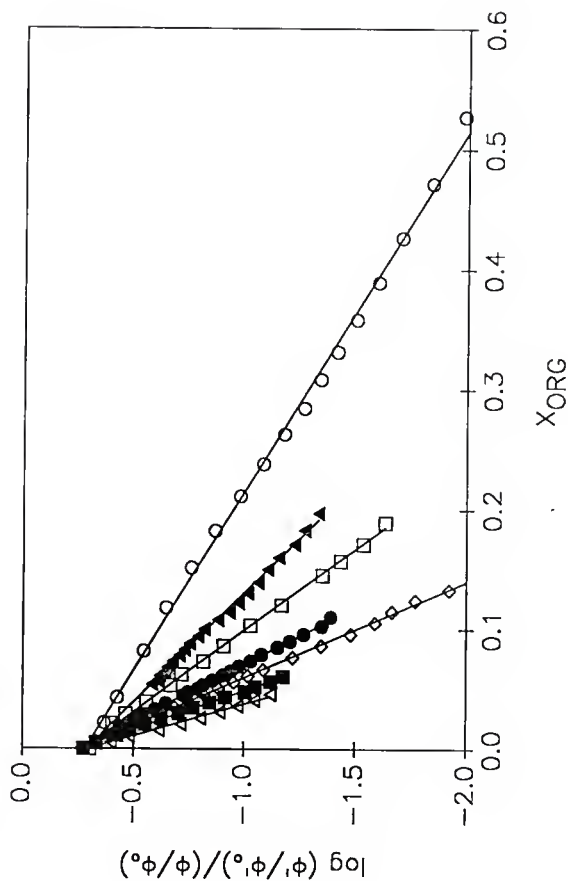


Figure 3.20. Plots of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ as a function of the organic cosolvent mole fraction (X_{ORG}) for 6-methoxyquinoline, where methanol -○-, ethanol -□-, isopropanol -◇-, t-butanol -■-, acetone -●-, dioxane -△- and formamide -▲- are X_{ORG} .

Table 3.8. Results of the regression analysis of the plots of $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ vs the mole fraction of organic cosolvent of 6-methoxyquinoline.

Cosolvent	Intercept	$(k\tau_0)_0$	Slope	r^2
methanol ¹	-0.28 ± 0.02	0.52	-3.2 ± 0.1	0.998
ethanol ¹	-0.27 ± 0.01	0.53	-7.4 ± 0.1	0.999
i-propanol ²	-0.26 ± 0.01	0.54	-12.7 ± 0.1	1.000
t-butanol ²	-0.25 ± 0.01	0.56	-15.5 ± 0.1	0.998
acetonitrile ¹	-0.26 ± 0.01	0.54	-10.3 ± 0.1	0.997
formamide ¹	-0.26 ± 0.01	0.54	-5.5 ± 0.1	0.997
dioxane ²	-0.28 ± 0.01	0.52	-17.9 ± 0.1	0.987

1. Results obtained from this investigation.
2. Results obtained through the reevaluation of Kelly's investigations (151).

and 3.22, the rate of acid dissociation was also found to decrease in a biexponential fashion. The slopes and intercepts obtained from the nonlinear least squares fitting of the data are listed in Table 3.9. Unlike 6MQ, one finds that the terminal slopes (m_2) are significantly different from zero. This suggests that proton transfer is still occurring at higher mole fractions of organic cosolvent. Further, the fact that the terminal slopes are approximately the same, implies that proton transfer is no longer dependent upon the identity of the organic component. Similar behavior can be found in Kelly's mixed solvent

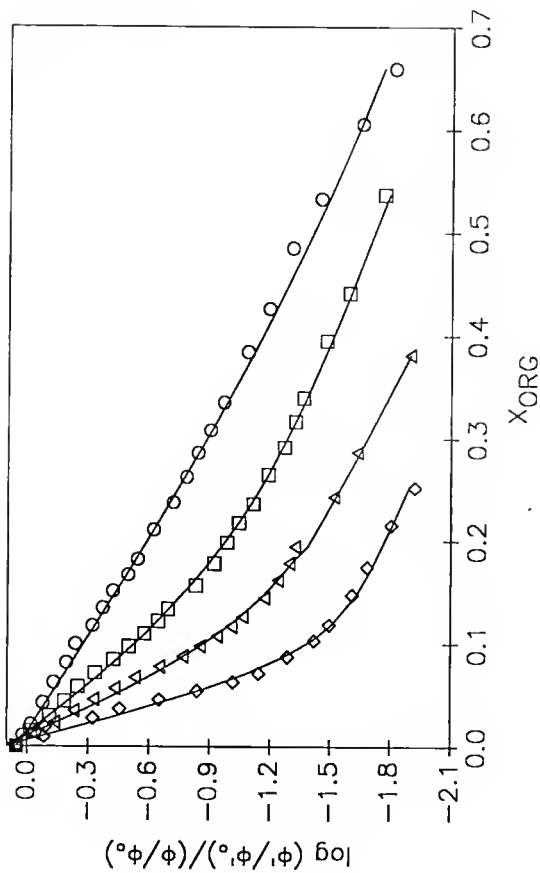


Figure 3.21. Plots of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ as a function of the organic cosolvent mole fraction (X_{ORG}) for 2-hydroxybiphenyl, where methanol -O-, ethanol -□-, isopropanol -Δ- and tertiary butanol -◇- are X_{ORG} .

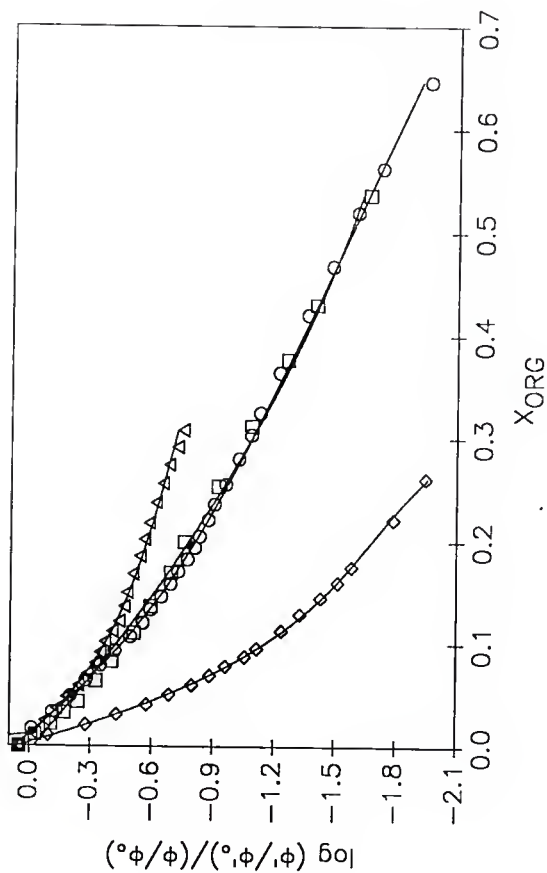


Figure 3.22. Plots of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ as a function of the organic cosolvent mole fraction for 2-hydroxybiphenyl acetonitrile -○-, dimethylsulfoxide -△-, dioxane -◇- and formamide -□-.

Table 3.9. Results of the biexponential fitting of the plots of $(\phi'/\phi'_0)/(\phi/\phi_0)$ versus mole fraction of organic cosolvent for 2-hydroxybiphenyl.

cosolvent	A_1	m_1	A_2	m_2
methanol	1.0 ± 0.1	-3.4 ± 0.3	0.08 ± 0.13	-1.3 ± 1.0
ethanol	1.1 ± 0.0	-7.5 ± 0.3	0.18 ± 0.02	-1.9 ± 0.1
isoprop.	1.1 ± 0.1	-10.7 ± 0.9	0.12 ± 0.07	-3.4 ± 0.8
t-but.	1.3 ± 0.1	-19.6 ± 1.2	0.05 ± 0.01	-2.2 ± 0.6
aceto.	0.8 ± 0.0	-8.1 ± 0.4	0.40 ± 0.03	-2.4 ± 0.1
formam.	0.6 ± 0.0	-13.4 ± 0.6	0.58 ± 0.01	-2.7 ± 0.1
dioxane	1.0 ± 0.1	-17.7 ± 1.1	0.12 ± 0.06	-3.8 ± 1.7
DMSO	0.7 ± 0.0	-9.1 ± 0.6	0.46 ± 0.03	-1.3 ± 0.1

titrations of the weakly acidic, substituted naphthols (151). Further, analogous phenomena has been seen in the mixed solvent ion solvation investigations of Kestov (116) where the deviations from linearity are attributed to the existence of three distinct structural regions in the mixed solvent system. Perhaps these concepts can be used to explain the inhibitory effect that organic cosolvents have on the rate of proton transfer. After all, reaction rates in solution are generally considered to be directly related to the differential free energy of solvation of transition states and reactants (92). However, if changes in solvation and/or changes in the solvent structure are solely

responsible for the inhibition of proton transfer in a mixed solvent system, then one would envision that the inhibitory nature of organic cosolvents on both acids and bases should be the same. In the initial mole fraction range this is the case. For example, in table 3.10, one finds that the slopes obtained for 6MQ and 2HBP are essentially identical. However, at higher concentrations of organic cosolvent, 6MQ does not show any sign of prototropic activity in the excited singlet state. On the other hand, 2HBP does.

Perhaps the lack of prototropic activity seen with 6MQ, once the mole fraction of the organic cosolvent exceeds a particular limit, stems from the fact that organic solvents have been shown to increase the vibronic coupling between the close lowest excited electronic states ($n\pi'$ and $\pi\pi'$) of similar compounds (i.e., acridine and quinoline) relative to the degree of coupling in water (44-46). If this same phenomenon occurs with 6MQ, then the addition of organic cosolvents to an aqueous system should increase the probability of nonradiative decay or singlet to triplet intersystem crossing processes for this molecule. Such effects would be reflected in both the steady-state and time-resolved studies because these processes depopulate the lowest excited singlet state during its lifetime.

The concept of increased vibronic coupling of 6MQ is supported by current time-resolved measurements and a recent

Table 3.10. A comparison of the inhibitory values; m , obtained from the linear regression analysis of $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ for 6-methoxyquinoline and m_1 , obtained from the biexponential fitting of $(\phi'/\phi'_0)/(\phi/\phi_0)$ for 2-hydroxybiphenyl as a function of the organic cosolvent mole fraction.

Cosolvent	m	m_1
methanol	-3.2 ± 0.1	-3.4 ± 0.3
ethanol	-7.4 ± 0.1	-7.5 ± 0.3
i-propanol	-12.7 ± 0.1	-10.7 ± 0.9
t-butanol	-15.5 ± 0.1	-19.5 ± 1.2
acetonitrile	-10.3 ± 0.1	-8.1 ± 0.4
formamide	-5.5 ± 0.1	-13.4 ± 0.6^1
dioxane	-17.9 ± 0.1	-17.7 ± 1.1

1. The inhibitory effect of formamide on 2HBP was deemed to be in error because the fluorescence from 2HBP was quenched by formamide. However a review of Kelly's data on the substituted naphthols in aqueous formamide indicated that the slope was 4.9 ± 0.4 , which is consistent with the results obtained for 6MQ (151).

solvation study of 6MQ in various organic solvents (153).

For example, it is seen that the lifetime in the absence of proton transfer of neutral 6MQ is approximately 5, 15 and 50% less in methanol, ethanol and acetonitrile than it is in water. Yet, in similar studies of 2HBP the lifetime of its neutral species, in the absence of proton transfer,

increases by a factor of 10. Further, from steady-state titrations, one discovers that as soon as the primary water structure of the mixed solvent system is destroyed, which graphically is depicted by the deviations from linearity in the $\log(\Phi'/\Phi'_0)/(\Phi/\Phi_0)$ versus mole fraction plots, the spectral changes which are associated with prototropic activity in the lowest excited singlet state of 6MQ are lost. This is not the case with 2HBP. Thus, evidence seems to suggest that the lack of prototropic activity in the terminal phase of the biexponential mole fraction plots of 6MQ, probably results from an increase in the vibronic coupling between the lowest excited singlet states of 6MQ which occurs concurrently with the increase in the mole fraction of organic cosolvent. However, since the structure of the mixed solvent system changes abruptly once water has become saturated with the organic cosolvent, then it is also possible that the cessation of prototropic activity stems from changes in the solvent structure. At this time it is impossible to tell which argument is the most plausible. Perhaps both processes influence the excited state behavior of 6MQ.

It is generally accepted that organic cosolvents become incorporated into the tetrahedral structuring of water, at least up until the concentration of organic cosolvent which correlates with the maximum stabilization of water structure

(119). From figure 3.21 one can speculate that the initial linear range coincides with or relates to the preservation of water structure. It can also be claimed from figure 3.21 that the structural integrity of water appears to be disrupted to a greater extent by the larger solvents. This becomes apparent when the slopes obtained from the alcohol series, listed in table 3.10, are plotted against their molecular volume as depicted in figure 3.23.

In solvation, water orders itself around the organic cosolvent molecule. This behavior is attributed to the formation of ice-like water cages or clusters (100,101). Thus, the addition of organic cosolvents to water can be considered to be structure making. Continued addition of organic cosolvent molecules to water, however, eventually leads to the destruction of these initial clusters of water. Therefore, addition of organic cosolvents to water is also structure breaking. One might speculate, at this point, that the remaining water molecules become less ordered once the primary water clusters are destroyed. However, recent evidence suggests that smaller more rigid clusters of water arise from the larger ones. Further, it seems that these smaller clusters tend to maintain their structural integrity over a wide range of mole fraction because the organic cosolvent will fill the newly formed voids and/or interstitial spaces that emerge with the destruction of the

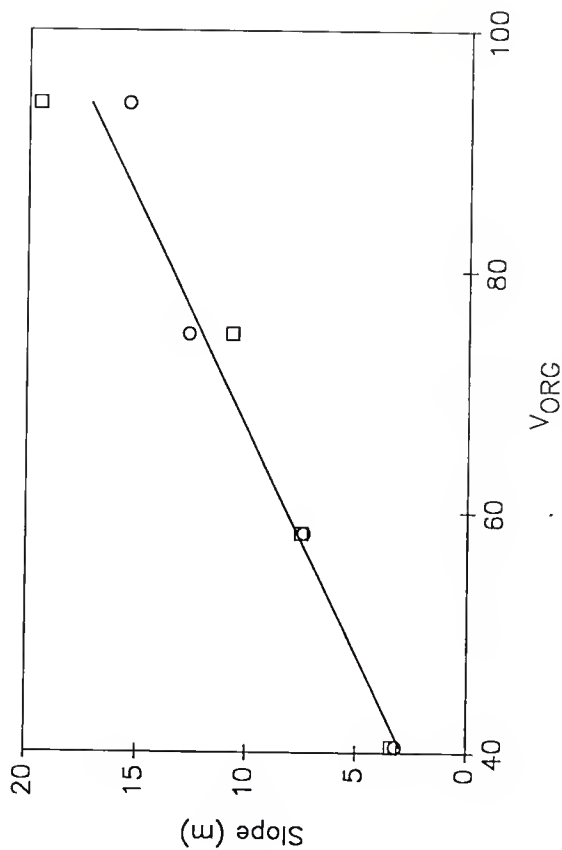


Figure 3.23. The slopes (m and m_1) obtained for 6-methoxyquinoline (O) and 2-hydroxybiphenyl (□) in aqueous alcohol as a function of the molecular volume (V_{ORG}) of alcohol.

$$\begin{aligned} V_{\text{ethanol}} &= 58.23 \text{ mg/ml} \\ V_{\text{t-butanol}} &= 93.98 \text{ mg/ml} \\ V_{\text{isopropanol}} &= 74.81 \text{ mg/ml} \\ V_{\text{methanol}} &= 40.48 \text{ mg/ml} \end{aligned}$$

primary clusters (172). These concepts help to explain how tetramers (112) and/or pentamers (113) of organic cosolvent begin to dominate liquid structure. They also help to explain how proton transfer continues to take place, especially since weak organic acids ($pK_a > 0$) do not dissociate measurably during the lifetime of the excited state unless an extended hydrogen bonded network of water is present within the mixed solvent system (9). Further, it can be inferred that both the size and prototropic capabilities of the residual water clusters must be the same because the terminal slopes of the $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ versus mole fraction plots, as depicted in figure 3.21, are similar.

It has long been recognized that the structure of water becomes rigidified on the nanosecond timescale following the addition of an organic cosolvent to water (80). According to theory, this process (i.e., the retardation of the molecular motions of the solvent) should have a strong effect on charge-transfer reactions (173). Such effects have been observed both for excited state reactions (174) and for dyes which change shape upon excitation (175). Recently, the rise time of excited state proton transfer luminescence of the conjugate species of 1-naphthol in water (33 ps) was found to be similar to the Debye relaxation time of the water (10 ps) (176). Since the rise time is considered to represent the rate of conversion of the neutral species to the

naphtholate anion and the solvated proton, solvent rigidity must play a role in excited state proton transfer (176). In fact it has been suggested by Robinson and coworkers (48), that proton transfer, a process that takes place on the subnanosecond timescale, is both kinetically dependent upon and limited solely by solvent relaxation. Unfortunately, these rapid relaxational motions lie beyond the scope of current technology. Therefore, the actual mechanism of proton transfer remains to be seen.

In an attempt to better understand proton transfer and its accompanying solvation, two macroscopic models will be discussed. These models are based upon the fact that reaction rates in solution are directly related to the differential free energy of solvation of transition states and reactants. The first model is based upon the concept of a "sphere-of-action" and can be derived as follows. If the lifetime of the reactant (acid or base) in the absence of proton transfer, τ_o , is assumed to be constant within the mixed solvent range where the structural integrity of water is maintained, then it follows that equation (1.9) can be reduced to:

$$k/k_o = e^{2.303 m X_{org}} \quad (3.13)$$

where k and k_o are the pseudo-first order rate constants for

photodissociation and photoprotonation of weak excitable acids and bases in mixed solvent and pure water, respectively. Further, if it is assumed that there is a sphere of volume in a mixed solvent system within which the organic cosolvent will completely inhibit the proton transfer reaction, the probability of immediately quenching the reaction is unity. Intuitively, if an organic cosolvent is within a reactant's "sphere-of-action", i.e., that range of water required to solvate the transferring proton, then proton transfer will not occur.

From the Poisson probability distribution (177), the probability P , of finding a volume with n molecules of organic solvent is:

$$P(n) = (\lambda^n / n!) e^{-\lambda} \quad (3.14)$$

where λ is the mean number of organic cosolvent molecules per volume of solvent, v . If $(4/3)\pi R^3$ is used to express the volume of the hydration "sphere-of-action" and the partial molar volumes of the mixed solvent system are assumed to be constant, as in ideal solutions, then the number of organic cosolvent molecules that enter into the sphere at a given concentration of mixed solvent can be given as the product of the mole fraction of organic cosolvent and the ratio of the volume of the hydration "sphere-of-action" to the molecular volume of the organic cosolvent as follows:

$$\lambda = \frac{(4/3)\pi R^3 N_0 X_{org}}{V_{org}} \quad (3.15)$$

where N_0 is the Avogadro's number and V_{org} is the partial molar volume of the organic cosolvent, which has been taken as a constant.

Now, if k/k_0 , the ratio of the pseudo-first order rate constants in mixed solvent and pure water, is taken as the probability that no organic cosolvent will enter the reactant's hydration "sphere-of-action", then when $n = 0$, it follows that:

$$k/k_0 = P(0) = e^{-\lambda} \quad (3.16)$$

Thus, substitution of equations (3.12) and (3.15) into equation (3.16), gives:

$$e^{2.303 m X_{org}} = e^{-\{(4/3)\pi R^3 N_0 \frac{X_{org}}{V_{org}}\}} \quad (3.17)$$

from which it follows that:

$$2.303 m = \frac{-(4/3)\pi R^3 N_0}{V_{org}} \quad (3.18)$$

Thus, the radius of the hydration "sphere-of-action" can be calculated. The values obtained for each of the mixed

solvent systems investigated are presented in table 3.11, below.

Table 3.11. The hydration "sphere-of-action" radii of 6MQ and 2HBP in each of the mixed solvent systems investigated.

Cosolvent	Radii 6MQ (Å)	Radii 2HBP (Å)
Methanol	4.91	5.01
Ethanol	7.33	7.36
Isopropanol	9.54	9.48
t-Butanol	11.00	11.87
Formamide	5.85	5.59
Acetonitrile	7.91	7.30
Dimethylsulfoxide	-	8.40
1,4-Dioxane	11.17	11.13

Notice that the radii values obtained for 6MQ and 2HBP in a particular mixed solvent are nearly identical. Furthermore, these values are reasonably close to 7.5 Å, which is generally taken to be the mean value for the interionic separation in an encounter complex or solvent separated ion-pair in a proton transfer reaction (65). Therefore, it is possible to interpret R in the case of the excited state protonation of a weak base as the minimum distance through which the proton must travel from a water molecule to an accepting 6MQ molecule, unimpeded by the

interruption of water structure in the vicinity of the base by the organic cosolvent. In turn, one may interpret R in the case of the excited state dissociation of a weak acid as the minimum distance through which the proton must travel from the acidic reactant to an accepting water molecule, unimpeded by the interruption of water structure in the vicinity of the acid by the organic cosolvent. Support for R comes from a plot of the "sphere-of-action" radii against the bulk dielectric constant for the series of alcohols investigated: the bulk dielectric constant, ϵ , is an indicator of how well the reaction medium can accommodate a charged species (see figure 3.24). Notice that a strong correlation is found, $r^2 = 0.995$.

According to Hubbard and Onsager (128), in the initial ionization of a reactant, as a proton migrates out into the solvent, the surrounding polarized fluid particles rotate according to the laws of hydrodynamics. Although dielectric relaxation tends to restore a polarization corresponding to the local field, the average delay is equal to the Debye relaxation time, τ_0 , which is the relaxation time for the orientation of solvent dipoles around the solvent separated ion-pair by the rapid rotational motions of water molecules. Therefore, R may also be defined as a coulombic sphere-of-influence. If the transferring proton moves beyond this sphere-of-influence in the lifetime of the excited state,

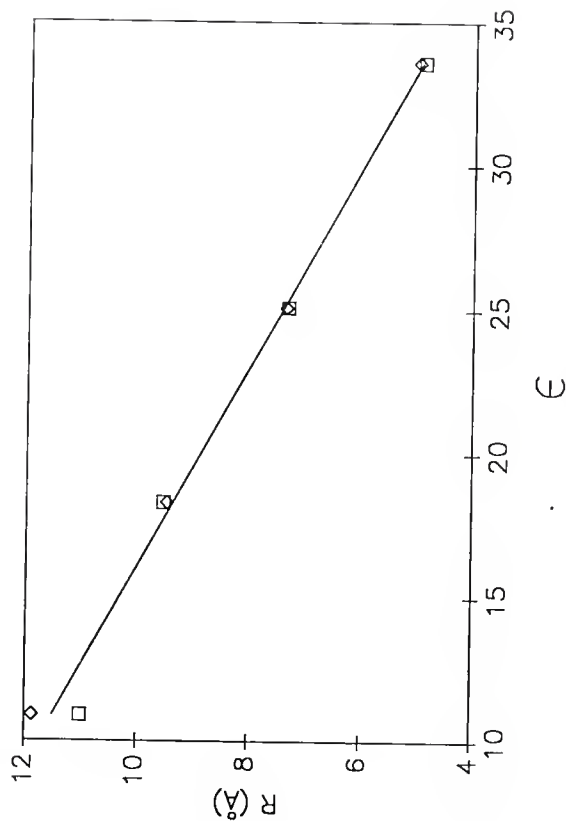


Figure 3.24.

The "sphere-of-action" radii (R), obtained for 6-methoxyquinoline ($\square$) and 2-hydroxybiphenyl ($\diamond$) in aqueous alcohol as a function of the bulk dielectric constant.

$$\begin{aligned} \epsilon_{\text{methanol}} &= 33.6 \text{ D} & \epsilon_{\text{ethanol}} &= 25.1 \text{ D} \\ \epsilon_{\text{isopropanol}} &= 18.3 \text{ D} & \epsilon_{\text{t-butanol}} &= 10.9 \text{ D} \end{aligned}$$

proton transfer occurs. However, if the proton remains within this sphere-of-influence in the lifetime of the excited state, then the ionizing reactant recombines. Thus, proton transfer is suppressed.

In view of this interpretation, one might expect that r , the difference between the hydration numbers of the ion-pair and the reactant products of the excited state reactions which are depicted in Cases 1 and 2, might actually be broadened in its interpretation to include all water molecules within a sphere of radius R around the dissociating acid or hydrolyzing base. Presumably, these water molecules would be more or less permanently oriented in the primary, secondary and even tertiary solvation shells of the products of the reaction. Support for this concept of hydration and/or solvation lie in the transition-state approach to excited state proton transfer (74,75).

At neutral pH, i.e., when the relative fluorescence intensities of the excited state reactants and products become constant or independent of pH, the following expression has been developed to quantitate excited state activity in aqueous-organic solvent systems (151):

$$\log \frac{(\phi' / \phi'_0)}{(\phi / \phi_0)} = \log(k_0 \tau_0) + r \log(a_w) - \log(k \tau_0) \quad (3.19)$$

where the slope r is considered to be the number of water molecules which are involved in the hydration of the proton during the transfer process and a_w is recognized to be the activity of water. All other terms have been previously defined. It is important to note that the lifetime of the excited state reactant in the absence of proton transfer was assumed to be constant with respect to the activity of water (151). Further, equation (3.19) and the "sphere-of-action" equations developed above are valid only in the range where the structural integrity of water is maintained.

From preliminary data of some hydroxyaromatic compounds in aqueous ethanol (75), a plot of $\log(k\tau_0)$ against $\log(a_w)$ was shown to be linear up through a mole fraction of approximately 0.2. Such behavior corresponds nicely with the initial aqueous region of a mixed solvent system as discussed earlier. Further, from linear regression analysis, the slope r was found to be 12.4 which suggests that 12 molecules of water participate in the reaction. A much smaller value of r (3 or 4) would be expected if only waters of hydration, i.e., water molecules in the primary hydration spheres of the reactants and products, were involved in the rate limiting step.

One might presuppose from above that the study of excited state proton transfer through the transition state (i.e., hydration) approach provides the means to determine

how many water molecules arrange themselves around the transferred proton in the aqueous phase of a mixed solvent system during its transfer from reactant to water or vice versa. One can even speculate that this approach provides a means to determine how much free water is available in the mixed solvent system to support the proton transfer process as organic cosolvents generally tie up and/or structure free water.

To test this hypothesis, the activities of water as a function of organic cosolvent mole fraction in each of the mixed solvent systems investigated (see appendix 1 for the published values) were obtained from the literature (179-185). In this way, $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ could be plotted against the $-\log(a_w)$, as depicted in figures 3.25-3.28. From the slopes of these linear plots, which have been compiled in table 3.12, one finds that the value of r increases with the ability of the cosolvent to structure water. For example, methanol does not structure water molecules as well as the larger cosolvents because its size and/or its bulk enables it to fit easily into the voids and interstitial spaces of water. As a result, the integrity of water is maintained over a wide range of mole fraction relative to the other solvents as seen in figures 3.20 and 3.21. However, the addition of tertiary butanol to water leads to a major rearrangement of water molecules. First of

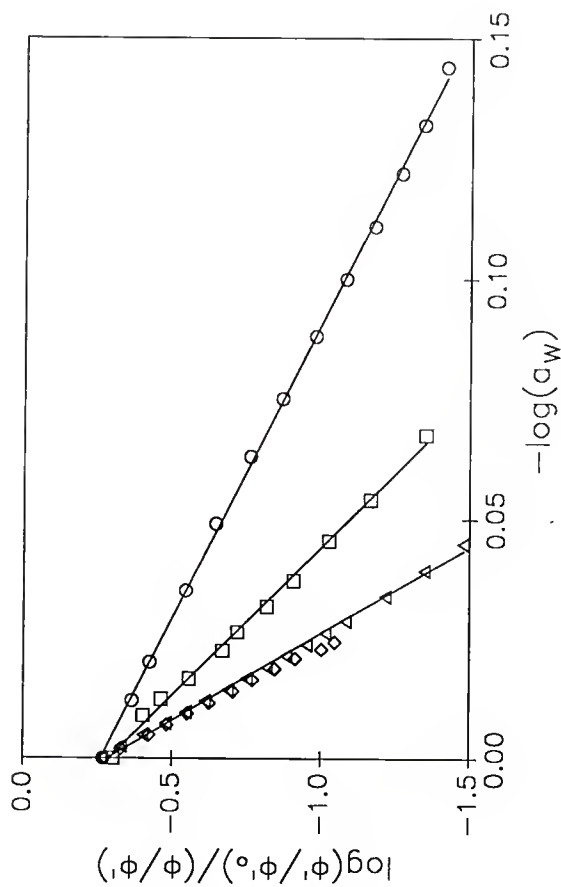


Figure 3.25. Plots of $\log(\phi'/\phi)/(\phi_0/\phi_0')$ as a function of the $-\log(a_w)$ for 6-methoxyquinoline in aqueous-methanol -O-, ethanol -□-, isopropanol -△- and tertiary butanol -◇-.

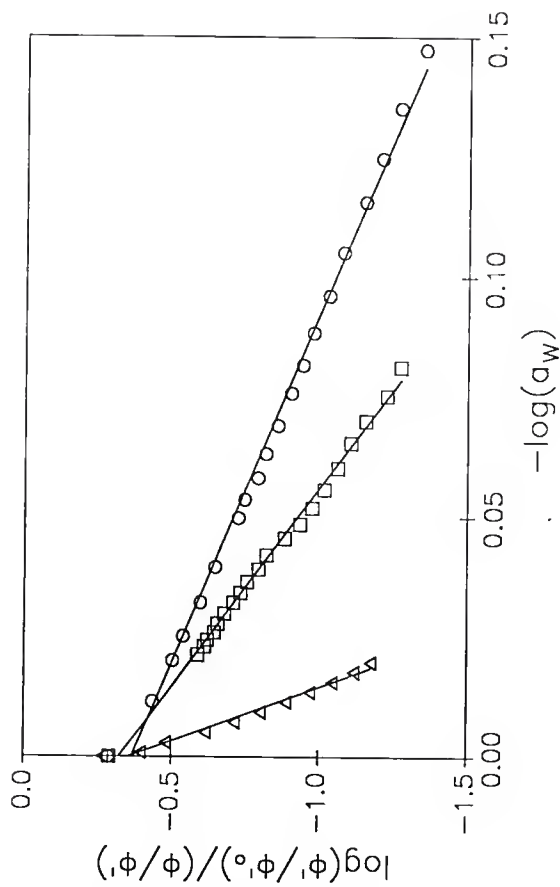


Figure 3.26. Plots of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ as a function of the $-\log(a_w)$ for 6-methoxyquinoline in aqueous-acetonitrile -O-, formamide -□-, and dioxane -△-.

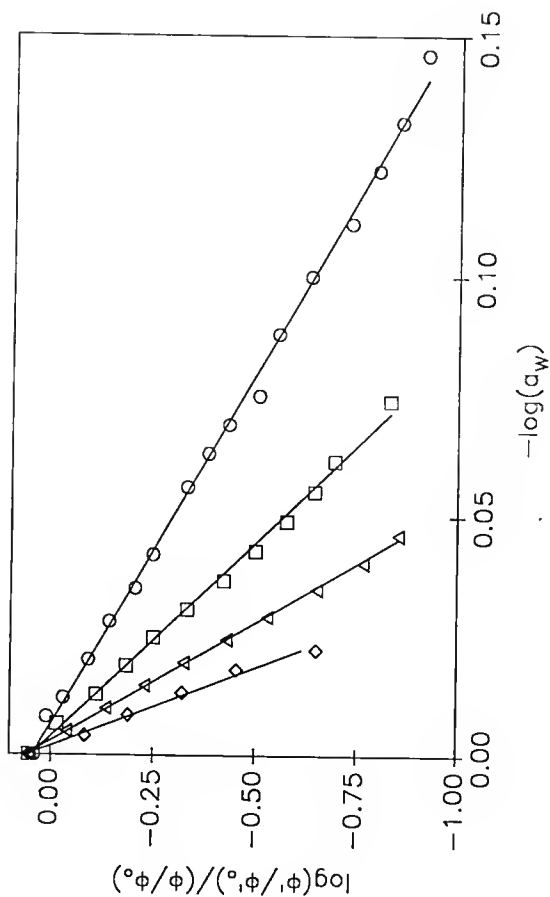


Figure 3.27. Plots of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ as a function of the $-\log(a_w)$ for 2-hydroxybiphenyl in aqueous-methanol -O-, ethanol -□-, isopropanol -Δ- and tertiary butanol -◇-.

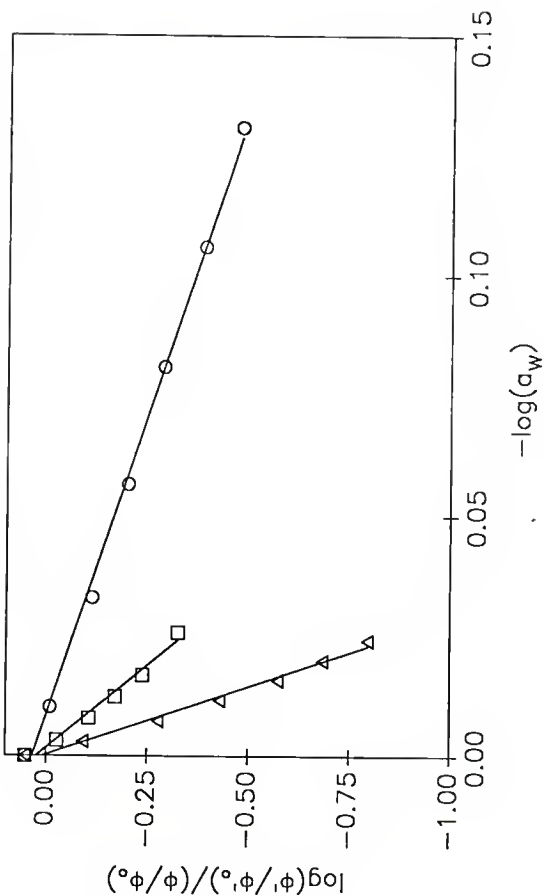


Figure 3.28. Plots of $\log(\phi'/\phi_0)/(\phi/\phi_0)$ as a function of the $-\log(a_w)$ for 2-hydroxybiphenyl in aqueous-acetonitrile -O-, formamide -□-, and dioxane -Δ-.

Table 3.12. Results of the regression analysis of the plots of $\log (\phi'/\phi'_o)/(\phi/\phi_o)$ vs $-\log (a_w)$ of 6-methoxyquinoline and 2-hydroxybiphenyl.

cosolvent	6-methoxyquinoline r	R ²	2-hydroxybiphenyl r	R ²
methanol	8.1 $\pm$ 0.1	0.999	8.0 $\pm$ 0.1	0.997
ethanol	16.1 $\pm$ 0.3	0.997	12.9 $\pm$ 0.1	0.999
i-propanol	28.1 $\pm$ 0.3	0.999	20.4 $\pm$ 0.2	0.999
t-butanol	31.6 $\pm$ 0.4	0.999	28.0 $\pm$ 0.6	0.999
acetonitrile	6.7 $\pm$ 0.1	0.997	4.0 $\pm$ 0.2	0.990
formamide	12.2 $\pm$ 0.2	0.994	16.8 $\pm$ 0.9	0.991
dioxane	44.6 $\pm$ 1.4	0.994	39.5 $\pm$ 1.5	0.996

all, its large size does not favor its incorporation into the voids and interstitial spaces of water. Second, its bulk does not readily allow it to approach molecules of water. Thus, more water is required to solvate tertiary butanol than methanol. This, in turn, leads to the formation of larger clusters of water (i.e., 28-32 water molecules, based upon r) for tertiary butanol versus smaller clusters of water (i.e., 8 water molecules, based upon r) for methanol. Similar behavior is found in the remaining mixed solvents investigated. Thus, it appears that the clustering of water represents the means by which water maintains its structural integrity. Support for the production of stable water

clusters can be found in the recent works of Castleman and his coworkers (137). In fact, this group has shown that the water clusters are capable of harboring migrating protons and/or hydroxide ions within their cage-like structures. This is not unlike Eigen's concept of rapid proton transfer (65). Thus, one might speculate that, if the proton is capable of navigating the dimensions of a preformed water cluster during the lifetime of the excited state, then the transition state approach to excited state proton transfer provides a means to determine the amount of water contained in the clusters which form subsequent to organic cosolvent addition. Keep in mind, however, that the lifetime of the neutral species in the absence of proton transfer is assumed to be constant. If this assumption fails, then the equations and perhaps even the macroscopic pictures of solvation which were developed from an interpretation of the data may be in error. Therefore, it is imperative that this assumption be challenged.

In an attempt to validate the assumption that the lifetime of the neutral species which undergoes proton transfer in the lowest excited singlet state remains constant throughout the entire organic cosolvent mole fraction range, time-resolved investigations were conducted. The fluorescence intensity of the acidic species of 2HBP was monitored in various aqueous-organic cosolvent mixtures as a

function of time. The resulting fluorescence decay profiles were found to be monoexponential and at neutral pH, conformed to equation (1.47):

$$I(\bar{v}, t)_A = I_A(\bar{v}, t = 0) e^{-\gamma_1 t} \quad (1.47)$$

where the observed rate constant, γ_1 , or inverse lifetime, τ^{-1} , is given as follows:

$$\gamma_1 = \tau^{-1} = \left(\frac{1}{\tau_0} + k_a \right) \quad (3.20)$$

It can be seen in figures 3.29 and 3.30, that the observed rate constant decreases in a biexponential manner when plotted as a function of the organic cosolvent mole fraction. This behavior is similar to that found in steady state experiments. In fact, when the data is fit with an expression similar to equation (3.12), the initial slope, m_1 , which depicts the inhibitory effect that organic cosolvents have on proton transfer in the aqueous range of a mixed solvent system, was found to be within two standard errors of the steady-state value (see tables 3.9 and 3.13 for a comparison). The one exception was aqueous formamide. However, since the steady-state emission of the neutral species of 2HBP is significantly quenched as the concentration of formamide is increased, an accurate determination of m^1 could not be obtained with any real

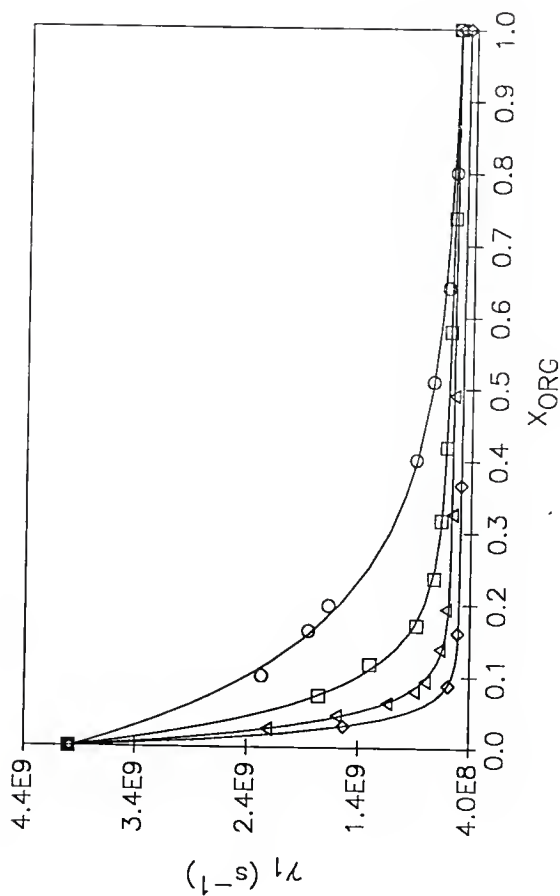


Figure 3.29. The observed rate constant (γ_1) as a function of the organic cosolvent mole fraction (X_{ORG}) for 2-hydroxybiphenyl in aqueous methanol -○-, ethanol -□-, isopropanol -△-, and t-butanol -◇-.

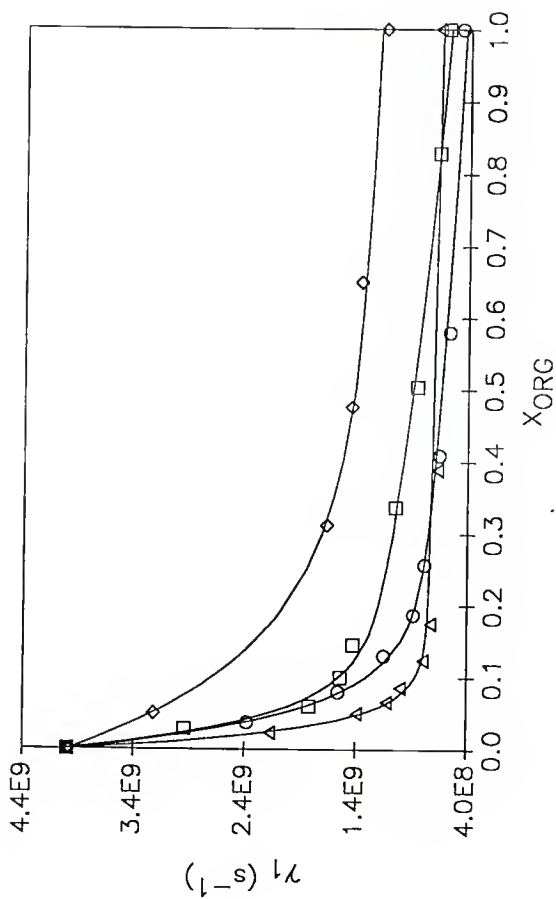


Figure 3.30. The observed rate constant (γ_1) as a function of the organic cosolvent mole fraction (X_{ORG}) for 2-hydroxybiphenyl in aqueous acetonitrile -O-, dimethylsulfoxide -□-, dioxane -Δ-, and formamide -◇-.

Table 3.13. Results of the biexponential fitting of the plots of γ_1 vs mole fraction of organic cosolvent for 2-hydroxybiphenyl.

cosolvent	$A_1 (\times 10^8 \text{s})$	m_1	$A_2 (\times 10^9 \text{s})$	m_2
methanol	3.2 ± 0.3	-2.8 ± 0.3	7.3 ± 2.2	-0.14 ± 0.16
ethanol	3.3 ± 0.0	-6.6 ± 0.3	6.9 ± 0.5	-0.10 ± 0.04
isoprop.	3.4 ± 0.0	-12.4 ± 0.2	5.9 ± 0.2	-0.04 ± 0.03
t-but.	3.5 ± 0.0	-17.7 ± 0.2	5.1 ± 0.1	-0.04 ± 0.01
aceto.	3.1 ± 0.0	-8.3 ± 0.4	9.3 ± 0.6	-0.32 ± 0.05
formam.	2.5 ± 0.1	-3.1 ± 0.2	15.2 ± 0.7	-0.10 ± 0.03
dioxane	3.2 ± 0.0	-14.8 ± 0.8	7.8 ± 0.2	-0.08 ± 0.06
DMSO	2.7 ± 0.0	-9.8 ± 1.8	14.0 ± 2.0	-0.39 ± 0.09

degree of certainty (see table 3.10). Thus, these results should probably be ignored.

The consistency between the initial slopes of the $\log(\phi'/\phi'_0)/(\phi/\phi_0)$ and γ_1 versus mole fraction plots provides support for the concept that inhibition of proton transfer is intimately related to an alteration of water structure. The terminal slopes, m_2 , seem to contradict the previous claim, that proton activity can still be observed after the structural integrity of water has been destroyed. This is because the time-resolved slopes approaches zero as the mole fraction of organic cosolvent approaches 1. Recall however,

that the steady-state slopes do not. This inconsistency between the experimental techniques is easily dispelled if one recognizes that the solvation of large polar organic molecules correlates well with the dielectric relaxation times of bulk solvent (174). In other words, once water becomes saturated with the organic cosolvent, the bulk solution better approximates the organic component, and so does the lifetime of 2HBP. It would, therefore, seem that the sensitivity of the time-resolved technique is lost once the primary structural integrity of water is destroyed. This is not the case with the steady-state technique because it responds to the acid-base properties of the fluorescent probe. Further, since excited state proton transfer does not take place in pure organic solvents and prototropic activity can still be found with this technique after water becomes saturated with the organic cosolvent, the evidence suggests that small, structurally intact clusters of water must remain within the mixed solvent system.

The observed lifetime (τ) of neutral 2HBP in mixed solvents are listed in table 3.14. One finds that τ increases proportionally with the mole fraction of organic cosolvent. Such results seem to suggest that the lifetime of 2HBP in the absence of proton transfer (τ_0) may also change with the concentration of organic cosolvent.

To challenge this concept, equation (3.20) was

Table 3.14. The lifetime of neutral 2-hydroxybiphenyl as a function of organic cosolvent mole fraction.

MeOH		EtOH		i-PrOH		t-BuOH	
X	τ (ns)	X	τ (ns)	X	τ (ns)	X	τ (ns)
0.000	0.25 $\pm$ 0.03	0.000	0.25 $\pm$ 0.03	0.000	0.25 $\pm$ 0.03	0.000	0.25 $\pm$ 0.03
0.100	0.44 $\pm$ 0.01	0.054	0.37 $\pm$ 0.02	0.026	0.45 $\pm$ 0.02	0.050	1.04 $\pm$ 0.03
0.163	0.54 $\pm$ 0.01	0.073	0.57 $\pm$ 0.01	0.045	0.63 $\pm$ 0.02	0.088	1.69 $\pm$ 0.03
0.198	0.60 $\pm$ 0.01	0.117	0.77 $\pm$ 0.01	0.063	0.88 $\pm$ 0.01	0.161	1.96 $\pm$ 0.02
0.401	1.11 $\pm$ 0.01	0.171	1.13 $\pm$ 0.01	0.080	1.11 $\pm$ 0.01	0.366	2.03 $\pm$ 0.03
0.510	1.33 $\pm$ 0.01	0.236	1.37 $\pm$ 0.01	0.094	1.25 $\pm$ 0.01	1.000	2.18 $\pm$ 0.02
0.641	1.60 $\pm$ 0.01	0.317	1.49 $\pm$ 0.01	0.139	1.49 $\pm$ 0.01		
0.801	1.76 $\pm$ 0.01	0.419	1.60 $\pm$ 0.01	0.194	1.62 $\pm$ 0.01		
1.000	1.81 $\pm$ 0.01	0.580	1.67 $\pm$ 0.01	0.326	1.76 $\pm$ 0.01		
		0.738	1.75 $\pm$ 0.01	0.491	1.78 $\pm$ 0.01		
		1.000	1.82 $\pm$ 0.02	1.000	1.86 $\pm$ 0.01		

Table 3.14--continued

ACN		DMSO		Diox.		Form.	
X	$\tau(\text{ns})$	X	$\tau(\text{ns})$	X	$\tau(\text{ns})$	X	$\tau(\text{ns})$
0.000	0.25 ± 0.03	0.000	0.25 ± 0.03	0.000	0.25 ± 0.03	0.000	0.25 ± 0.03
0.037	0.42 ± 0.01	0.028	0.34 ± 0.01	0.023	0.46 ± 0.01	0.050	0.31 ± 0.02
0.080	0.64 ± 0.01	0.060	0.55 ± 0.01	0.050	0.72 ± 0.01	0.312	0.60 ± 0.02
0.130	0.87 ± 0.01	0.100	0.65 ± 0.01	0.066	0.89 ± 0.01	0.476	0.69 ± 0.02
0.187	1.13 ± 0.01	0.145	0.70 ± 0.01	0.086	1.00 ± 0.01	0.650	0.73 ± 0.02
0.257	1.27 ± 0.01	0.203	0.71 ± 0.01	0.124	1.26 ± 0.01	1.000	0.83 ± 0.02
0.409	1.52 ± 0.02	0.337	0.95 ± 0.01	0.175	1.37 ± 0.01		
0.580	1.75 ± 0.01	0.504	1.16 ± 0.01	0.389	1.49 ± 0.01		
1.000	2.10 ± 0.01	0.829	1.48 ± 0.01	1.000	1.48 ± 0.01		
		1.000	1.67 ± 0.01				

rearranged to obtain the following expression.

$$\tau = \frac{\tau_0}{1 + k_a \tau_0} \quad (3.21)$$

In pure water, the values of k_a and τ_0 are $21 \times 10^8 \text{ s}^{-1}$ and $0.53 \times 10^{-9} \text{ s}$, respectively (see table 3.5 above). With their substitution into equation (3.21); $\tau = 0.25 \times 10^{-9} \text{ s}$ which is consistent with its experimentally obtained value in water. Further, since one finds that τ increases to a maximum value of $\approx 2.0 \times 10^{-9} \text{ s}$ at $X_{\text{ORG}} = 1$ in table 3.14, it is easily reasoned from equation (3.21) that τ_0 must also increase as k_a decreases. In fact, τ must be equal τ_0 at $X_{\text{ORG}} = 1$, since proton transfer does not occur in pure organic solvent (i.e., $k_a \rightarrow 0$). From this, one can claim that the assumption of a constant lifetime in the absence of proton transfer with increasing amounts of organic cosolvent is not always true. Thus, it is important to develop a means to obtain reasonable estimates of τ_0 .

A reasonable estimate of τ_0 can be obtained by combining steady-state equation (1.18):

$$\frac{\phi' / \phi'_0}{\phi / \phi'} = k_a \tau_0 \quad (1.18)$$

with time-resolved equation (3.20) and by making the

appropriate substitutions to obtain the following expression:

$$\tau_0 = \frac{1 + \frac{\phi'/\phi'_0}{\phi/\phi_0}}{\gamma_1} \quad (3.22)$$

This equation, which utilizes data obtained with both techniques, was subsequently verified in aqueous methanol and aqueous ethanol through the following relationship (141):

$$\tau_0 = \frac{\tau}{\phi/\phi_0} \quad (3.23)$$

where ϕ/ϕ_0 represents the relative quantum yield of neutral 2HBP in the same mole fraction of organic cosolvent in which the lifetime has been obtained. As can be seen in figure 3.31, there is reasonable correlation between estimates of τ_0 obtained with both methods. Therefore, equation (3.22) was utilized to provide the necessary estimates of τ_0 .

The relative quantum yield ratio $(\phi'/\phi'_0)/(\phi/\phi_0)$ obtained at steady-state in the mixed solvent systems investigated were divided by the τ_0 values generated with equation (3.22) to secure estimates of the dissociation and/or forward rate constant, k_a . When these values are

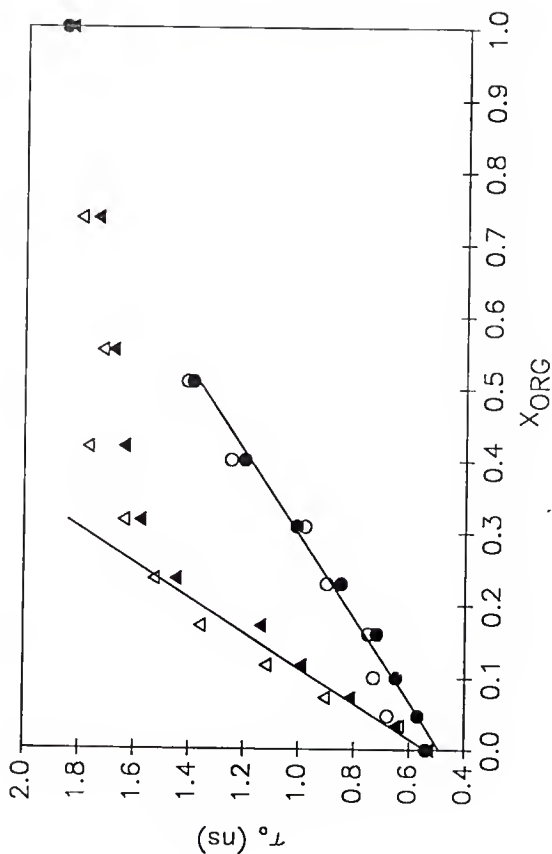


Figure 3.31. Lifetime of 2-hydroxybiphenyl in the absence of proton transfer against the organic cosolvent mole fraction (X_{ORG}) in aqueous methanol -O- and aqueous ethanol -Δ-, based upon equation (3.22). Solid circles and triangles were obtained from equation (3.23).

plotted against the mole fraction of organic cosolvent as depicted in figures 3.32 and 3.33, one finds that the logarithm of the dissociation rate constant also decreases in a biexponential fashion as the organic cosolvent mole fraction increases. The one exception is aqueous methanol. In this case, $\log(k_a)$ is observed to decrease linearly with the mole fraction of methanol. The reason for this behavior may lie in the fact that methanol does not alter the structural integrity of water as much as the other solvents do.

The dissociation rate constants were subsequently fit with an expression similar to equation (3.12). The results given in table 3.15 indicate that both the initial (m_1) and terminal (m_2) slopes are at least two to three times larger than those values obtained with the composite function (see Table 3.9). Further, the terminal slopes remain parallel and are within two standard errors of each other. This supports the concept that once the organic cosolvent destroys the structural integrity of water, the proton transfer inhibition rate no longer depends upon the type of organic cosolvent added to the system. Instead, evidence suggests that the inhibition of proton transfer becomes dependent upon small, but structurally, similar clusters of water which appear to remain intact over a wide range of the organic cosolvent mole fraction.

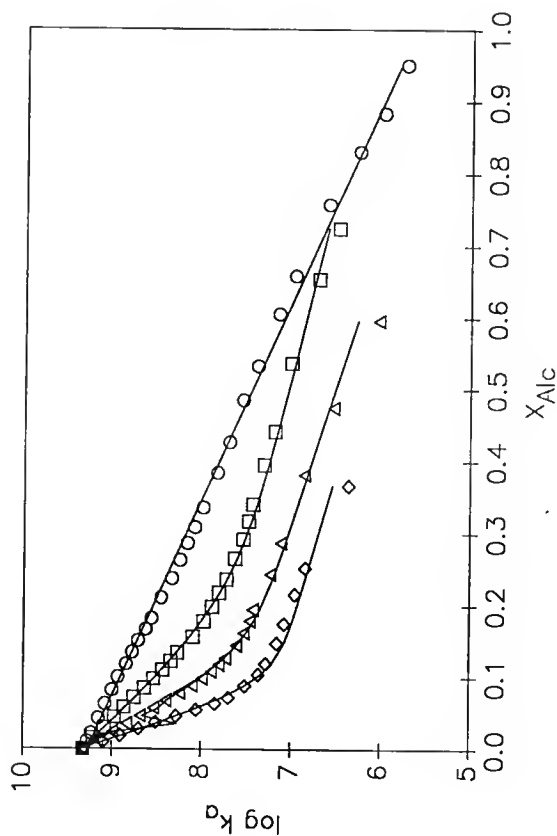


Figure 3.32. $\log(k)$ as a function of organic cosolvent mole fraction (X_{org}) for 2-hydroxybiphenyl in methanol -O-, ethanol -□-, isopropanol -Δ-, and tertiary butanol -◇-.

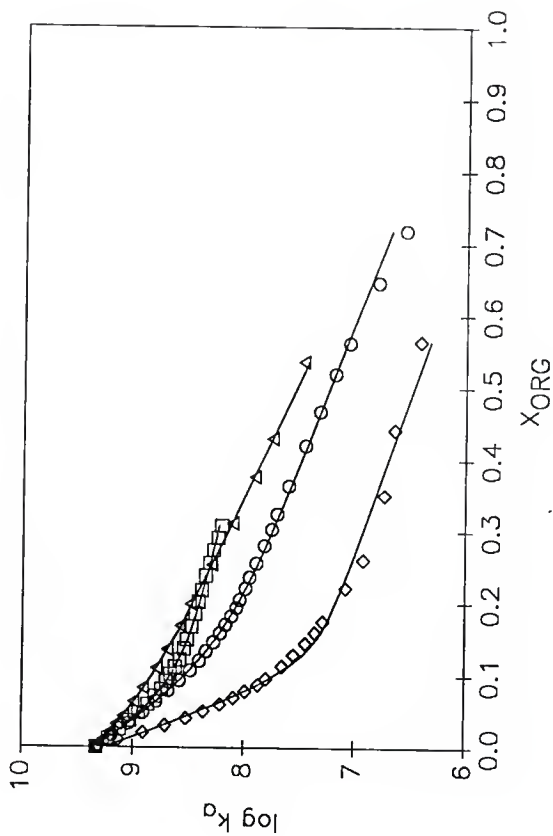


Figure 3.33. $\log(k)$ as a function of organic cosolvent mole fraction (X_{ORG}) for 2-hydroxybiphenyl in acetonitrile - $\circ$ -, dimethylsulfoxide - $\square$ -, formamide - Δ -, and dioxane - $\circ$ -.

Table 3.15. Results of the biexponential fitting of the plots of k_s vs the mole fraction of organic cosolvent for 2-hydroxybiphenyl.

cosolvent	$A_1 (\times 10^8 \text{s})$	m_1	$A_2 (\times 10^8 \text{s})$	m_2
methanol	21.1 ± 0.1	-9.3 ± 0.1		
ethanol	20.7 ± 0.2	-20.4 ± 0.3	1.0 ± 0.1	-4.5 ± 0.3
isoprop.	20.8 ± 0.2	-34.6 ± 0.5	0.6 ± 0.1	-5.9 ± 0.6
t-but.	21.1 ± 0.4	-54.7 ± 1.2	0.3 ± 0.1	-5.4 ± 1.8
aceto.	18.3 ± 0.2	-23.8 ± 0.4	3.2 ± 0.2	-5.8 ± 0.2
formam.	9.9 ± 0.7	-21.3 ± 1.1	11.3 ± 0.1	-7.0 ± 0.3
dioxane	17.6 ± 0.2	-40.9 ± 1.9	0.4 ± 0.1	-5.2 ± 0.4
DMSO	16.6 ± 0.2	-26.2 ± 0.4	4.8 ± 0.2	-3.4 ± 0.2

From the slopes listed in the table 3.15, the "sphere-of-action" radii for 2HBP were recalculated and compiled with the originally obtained results in table 3.16. Notice, that the new values no longer reflect the distance of interionic separation that is associated with an encounter complex or solvent separated ion-pair. Instead, they appear to reflect a Coulombic sphere-of-influence. In other words, if an organic cosolvent molecule enters this sphere prior to excitation, then proton dissociation and its accompanying solvation during the lifetime of the excited state is prevented.

If radii are generated from the terminal slopes (m_2)

Table 3.16. The hydration sphere-of-action radii of 2HBP in mixed solvent systems.

Cosolvent	Radii ¹ (Å)	Radii ² (Å)	Radii ³ (Å)
Methanol	5.01	13.73	-
Ethanol	7.36	20.02	4.41
Isopropanol	9.48	30.62	5.24
t-Butanol	11.87	33.12	3.26
Formamide	5.59	8.83	2.90
Acetonitrile	7.30	21.46	5.24
Dimethylsulfoxide	8.40	24.18	3.20
1,4-Dioxane	11.13	25.60	3.28

- 1) The radii listed in this column come from Table 3.11.
- 2) The radii listed in this column were derived using equation (3.19) with the values of m_1 given in Table 3.15.
- 3) The radii listed in this column were derived using equation (3.19) with the values of m_2 given in Table 3.15.

listed in Table 3.15, one finds that they are more reflective of the 7 Å generally taken to be the mean distance between solvent separated ion-pairs (see Table 3.16). In fact, from their consistency (range 4 ± 1 Å), one might consider that 4 Å represents the interionic separation of the 2HBP solvent separated ion-pair. To provide support for such concepts, one can turn to Coulomb's Law:

$$F = \frac{Q_1 Q_2}{4\pi\epsilon_0\epsilon r^2} \quad (3.24)$$

where F is the force between two charges Q_1 and Q_2 , r is the distance between these charges, ϵ is the dielectric constant of the medium, and ϵ_0 is the permittivity of free space.

It can be reasoned in a mixed solvent titration that the initial decrease in the polarity of the solvent (i.e., dielectric strength of the medium) can be attributed to the replacement of uncoordinated water molecules by organic cosolvent molecules, since uncoordinated water molecules are considerably more polar than coordinated ones. Further, it can also be reasoned that the charge which develops from the excited state ionization of a fluorescent probe can be easily dispersed when the composition of the mixed solvent system is predominantly aqueous in nature. Thus, Coulombic interactions, e.g., ion-ion and ion-dipole forces, tend to be relatively unimportant in solutions of high dielectric strength (> 30). However, once the water becomes saturated with organic cosolvent molecules, a highly structured microphase consisting predominantly of water in which all water molecules are highly ordered is produced. At this point, the charge produced during the process of ionization

is not easily dispersed. Further, if one speculates that the decrease in solvent polarity (dielectric strength of the medium) arises from disorder in the organic cosolvent molecules rather than the highly structured water, then one can easily imagine how the attractive Coulombic forces would tend to dominate the proton transfer process in this range. This also helps to explain the consistency in the observed radii obtained from the terminal phases of the mixed solvent plots.

The effect of dielectric strength on the rate of proton transfer is not a new one. Early on, Eigen (65) considered that the change in the rate constant for the diffusion controlled separation of an ion-pair with solvent composition in dilute solutions is inversely proportional to the accompanying change of the bulk dielectric constant. However, Huppert et al. (9,21) have since shown that the rate of proton dissociation is more sensitive to the change in solvent composition. Further, they reasoned, if proton transfer could be viewed as occurring from an initial state in which the proton is bound to the fluorescent probe and if the final state could be viewed as consisting of the anion of the fluorescent probe and a proton hydrated in a complex of r water molecules, then cosolvent addition to water affects the rate of proton transfer by altering the structure of water. Owing to the fact that the proton jumps

back and forth between the different water molecules of the complex at a very high rate of speed ($> 10^{12} \text{ sec}^{-1}$), the effective cross section for reactions involving H^+_{aq} should be determined by the extent of the entire hydrate complex, i.e., the solvent separated ion-pair (65).

According to this study, the initial sphere-of-action appears to coincide with Huppert's (9,21) claim that proton transfer is influenced predominantly by solvent structure. However, once the structural integrity of water is lost, the sphere-of-action radii obtained in each of the mixed solvent systems investigated approach a minimum. This minimum, $4 \pm 1 \text{ \AA}$, appears to be governed by the Coulombic interactions associated with the ionization process. Such observations tend to coincide with the predictions of Eigen. This suggests that the mechanism associated with the proton transfer process is changing; going from a translational-diffusion controlled regime (i.e., translation of water molecules to satisfy the local concentration requirement) to a rotational-diffusion controlled regime (i.e., rotational diffusion of water molecules to satisfy the local structural requirement).

This broader interpretation of R provides support for the notion that the slope, r from the transition state approach to excited state proton transfer represents all water molecules within a sphere of radius R around the

dissociating acid and/or hydrolyzing base. One might even suggest that r represents the total amount of water capable of supporting proton transport at a given mole fraction of organic cosolvent within a mixed solvent system. Presumably these molecules of water would exist in the form of clusters that shrink in size as the concentration of organic cosolvent is increased. Support for these concepts can be obtained from plots of $\log(k)$ versus $-\log(a_w)$, provided one assumes that the observed nonlinear behavior depicted in figures 3.34 and 3.35 relates to water's structural integrity and its ability to support the proton transfer process. It also provides the impetus to divide each of these plots up into linear segments and to surmise that they represent a range within the mixed solvent in which the structural integrity of water is constant. Further, if one considers that the free energy of the proton transfer reaction is primarily dependent upon the enthalpy of proton hydration and if one assumes that a linear relationship exists between the free energy of the reaction and proton transfer, equation (3.19) can be used as a tool to characterize the structuring of water over the entire mole fraction range. The results listed in Table 3.17 indicate that the amount of free water available to support the proton transfer process decreases as the concentration of organic cosolvent increases. In a sense, the sphere of

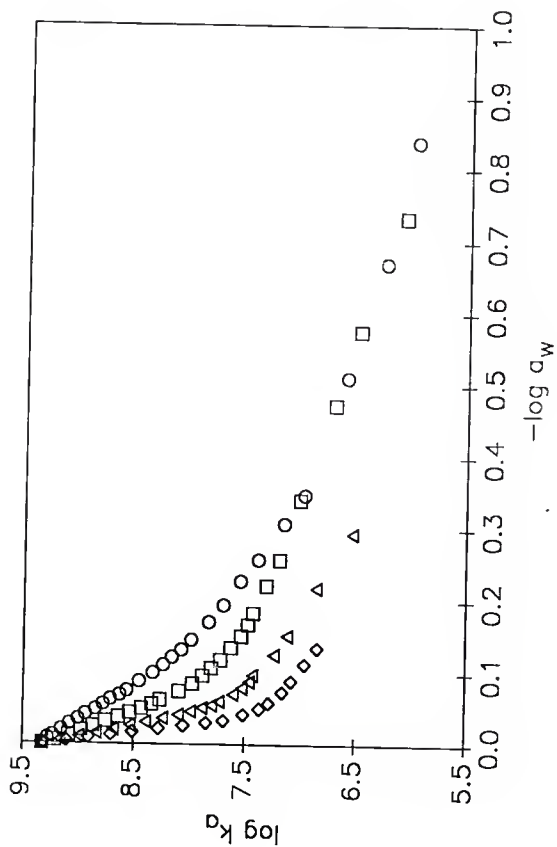


Figure 3.34. A plot of $\log(k)$ versus $-\log(a_w)$ in aqueous alcohol systems for 2-hydroxybiphenyl.
 $\square$ - ethanol
 $\circ$ - methanol
 $\triangle$ - isopropanol
 $\diamond$ - tertiary butanol

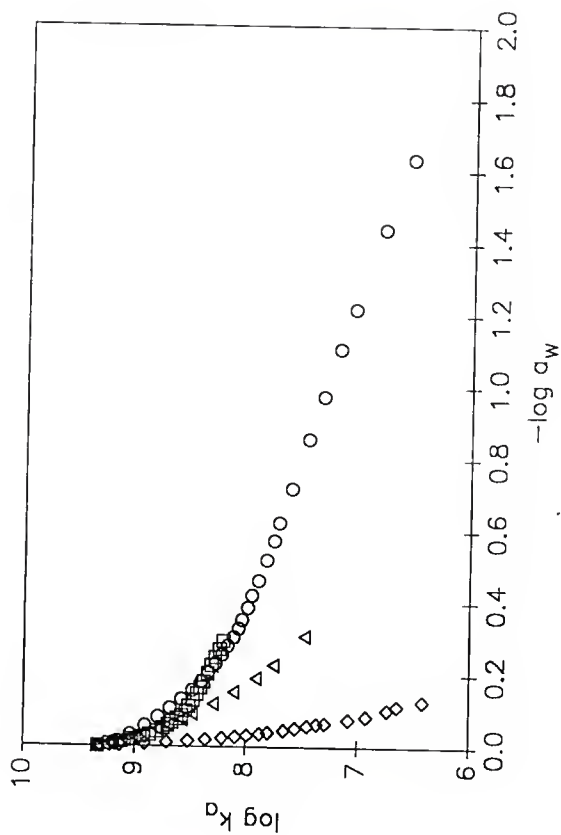


Figure 3.35. A plot of $\log(k)$ versus $-\log(a_w)$ in aqueous organic cosolvent systems for 2-hydroxybiphenyl.
 $\circ$ - acetone
 Δ - formamide
 $\square$ - dimethylsulfoxide
 $\diamond$ - dioxane

Table 3.17. Results of the linear regression analysis of the plots of $\log (k)$ versus $-\log (a_w)$ for 2-hydroxybiphenyl.

cosolvent	r_1	r_2	r_3
methanol	9.7 ± 0.2	4.9 ± 0.1	2.0 ± 0.1
ethanol	17.1 ± 0.2	5.0 ± 0.3	2.3 ± 0.1
isopropanol	29.2 ± 0.4	4.3 ± 0.1	
t-butanol	48.9 ± 0.6	5.5 ± 0.3	
acetonitrile	5.0 ± 0.1	1.1 ± 0.1	
formamide	14.0 ± 0.5	6.0 ± 0.5	
dimethylsulfoxide	14.7 ± 0.5	1.7 ± 0.1	
dioxane	48.0 ± 1.8	15.3 ± 0.7	

cluster of water around the dissociating acid and/or hydrolyzing base shrinks in size.

Kelly (51) has surmized, that if proton transfer could occur in water clusters of size differing from that cluster size which in solutions of unit activity of water is required for proton transfer, then, when the activity of water in a mixed solvent is reduced, a region of water activity should be reached in which the smaller cluster becomes the predominant accepting species. The data collected above appears to support this idea. For example, from Table 3.17 one finds that the organic cosolvents which were investigated cause water to be structured differently.

However, as the activity of water decreases, so does the size of the water cluster. Based upon the data compiled in Table 3.17, it appears that in the majority of the mixed solvents examined, the predominant proton accepting species is comprised of approximately 5 water molecule. These results tend to support Eigen's (37-39,62,66) theory of proton transfer and the claim made by Robinson (48) that acid dissociation and its attendant proton hydration produce the H_3O_4^+ ion as its direct kinetic product. However, one should note that the smallest proton accepting species in the methanol- ethanol- and dimethylsulfoxide-water systems appears to be 2 molecules of water. These results tend to support the concerted chain-like mechanism put forth by Conway (23, 136), and the idea that the mechanism of proton transfer changes concurrently with both the nature and composition of the solvent system changes (138).

In aqueous-dioxane it appears that a water cluster comprised of 15 molecules is the smallest proton accepting species, although the rationale for this is not clear. Further, in aqueous-acetonitrile, data suggests that one water molecule can support the proton transfer process. If this was the case, then the reaction would be too fast to follow on the nanosecond time-scale.

One possible explanation for deviations away from a 5-membered water cluster might lie in the fact that the

results presented above are dependent upon activity of water data taken from the literature. Therefore, the validity of activity data should be questioned before dismissing the concepts developed above.

Traditionally, the activity of water in mixed solvents are calculated from vapor pressure measurements on the basis of the Gibbs-Duhem equation and the assumption that gaseous mixtures are ideal (i.e., they adhere to Raoult's law). However, one should realize that seldom does the vapor phase above an aqueous organic mixture demonstrate ideal behavior. Both positive and negative deviations from Raoult's law can and do occur. Thus, the activities obtained in this manner could be in error. Water activity in mixed aqueous-organic solvent systems can also be obtained by using the colligative properties of the solvent. However, one can generally expect similar deviations from ideality. Thus, there should be no surprise to find that the activity data obtained by Goates and Sullivan (183) with the freezing point depression method for the aqueous-dioxane system correlate well with those determined from the measurement of vapor pressure.

Perhaps an alternative approach to determine water activity in a mixed aqueous-organic solvent system might be the spectroscopic investigation of excited state proton transfer. Huppert et al. (21) have already demonstrated that

the activity of water in concentrated electrolyte solutions could be determined by measuring the rate of excited state proton transfer from 2-naphthol and several derivatives to the solvent. Therefore, it seems logical that an evaluation of excited state activity in a mixed solvent system should also provide a measure of water activity, especially since the organic cosolvent does not support the proton transfer process.

If the most recent activity of water data for the aqueous alcohols is examined graphically as a function of the organic cosolvent mole fraction as shown in figure 3.36, one finds that water activity is linearly related to mole fraction. In fact, the slope obtained from each of the aqueous-alcohols is approximately one (see Table 3.18). Unfortunately, with the exception of aqueous-methanol, data is not available over the entire mole fraction range. However, since the terminal phases of the $\log(k\tau_0)$ and $\log(k)$ versus mole fraction of alcohol plots tend to be parallel (see figures 3.21 and 3.32), one can surmise that a unit of water activity within these mixed solvent systems has been reached because the inhibitory effect on the rate of proton transfer has become constant. In other words, the cosolvent no longer influences the reaction rate. From this, one can speculate that water activity governs the proton transfer process. Further, it seems logical to estimate

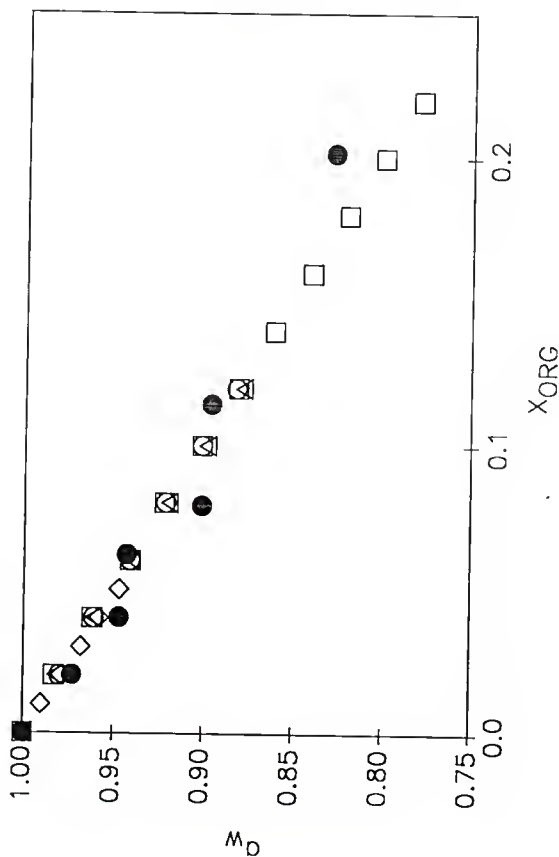


Figure 3.36. A plot of published water activity as a function of the organic cosolvent mole fraction, in -O- methanol (179, 180), -□- ethanol (181), -A- isopropanol (181) and -O- tertiary butanol (182)

Table 3.18. Linear regression analysis of the published water activity against the mole fraction.

solvent	intercept	slope	R ²
methanol ^a	1.00 ± 0.01	0.99 ± 0.01	0.999
	0.99 ± 0.01	0.82 ± 0.02	0.997
ethanol ^b	1.00 ± 0.01	1.02 ± 0.01	0.999
isopropanol ^c	1.00 ± 0.01	1.04 ± 0.02	0.999
tertiary butanol ^d	1.00 ± 0.01	1.06 ± 0.02	0.999

a) Linear mole fraction range is 0-0.7 (179,180).

b) Linear mole fraction range is 0-0.26 (181).

c) Linear mole fraction range is 0-0.22 (181).

d) Linear mole fraction range is 0-0.05 (182).

water activity in this range from the slopes provided in Table 3.18 and utilize this data as shown in figure 3.34. Support for the estimate of water activity comes from the fact that as the activity of water is reduced, the size of the proton accepting species (i.e., the water cluster) in each of the mixed aqueous-alcohol systems tends to be equivalent. In addition, results from the remaining solvent systems tend to coincide with those obtained with the alcohol series. Any observable differences probably stem from the interactions (i.e., the ones that lead to the deviations from Raoult's law) which occur between the

solvent and water. In other words, the observable differences may arise from the published activity data which has been generated from vapor pressure and other colligative property measurements.

The above results suggest that the excited state proton transfer technique can be used as a tool to calculate water activity. Additional support for this claim comes from the following study of solvent effects upon the reprotonation rate constant which demonstrates that this technique can be used to establish pH scale in mixed solvent systems.

Cosolvent Effects on the Reprotonation Rate Constant

The dependence of the relative quantum yields of fluorescence of 2-hydroxybiphenyl in solvents containing varying molar ratios of organic cosolvent and water on the formal hydrogen ion concentration $[H^+]$ are shown in figures 3.37 through 3.42. At formal hydrogen ion concentrations greater than 1×10^{-2} M, the anion is the sole absorbing and emitting species. In the intervals 1×10^{-3} M $> [H^+] > 1 \times 10^{-7}$ M and 1×10^{-6} M $> [OH^-] > 1 \times 10^{-8}$ M, the neutral molecule is the sole absorber. However, fluorescence is observed from the anion as well as from the neutral species. This results from dissociation in the lowest excited singlet state of the phenolic group of the directly excited neutral molecule (141). In this region of acidity or alkalinity, the

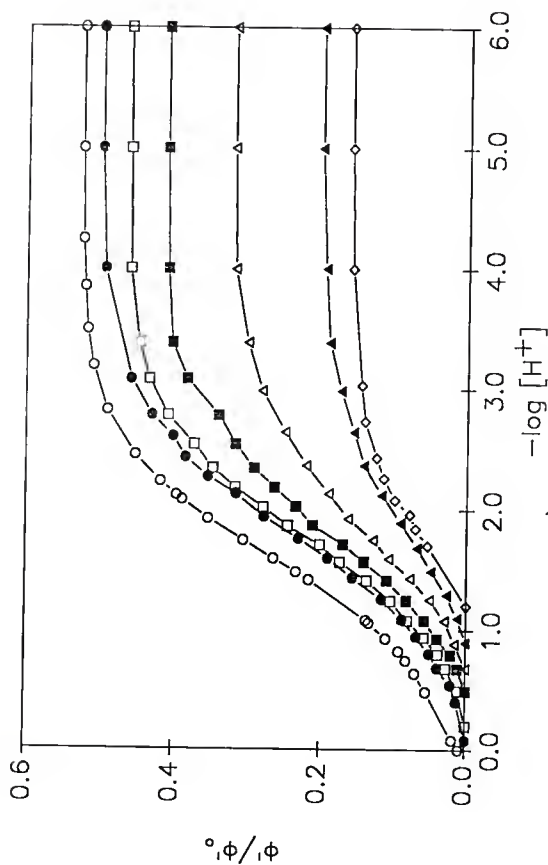


Figure 3.37. Variation of the relative quantum yields of fluorescence of the conjugate base (ϕ/ϕ_0) species of 2-hydroxybiphenyl with the formal hydrogen ion concentration $[H^+]$ in aqueous methanol. $-O-$ ($X_m=0.000$), $-●-$ ($X_m=0.100$), $-□-$ ($X_m=0.161$), $-■-$ ($X_m=0.229$), $-▽-$ ($X_m=0.308$), $-▲-$ ($X_m=0.401$), $-○-$ ($X_m=0.510$).

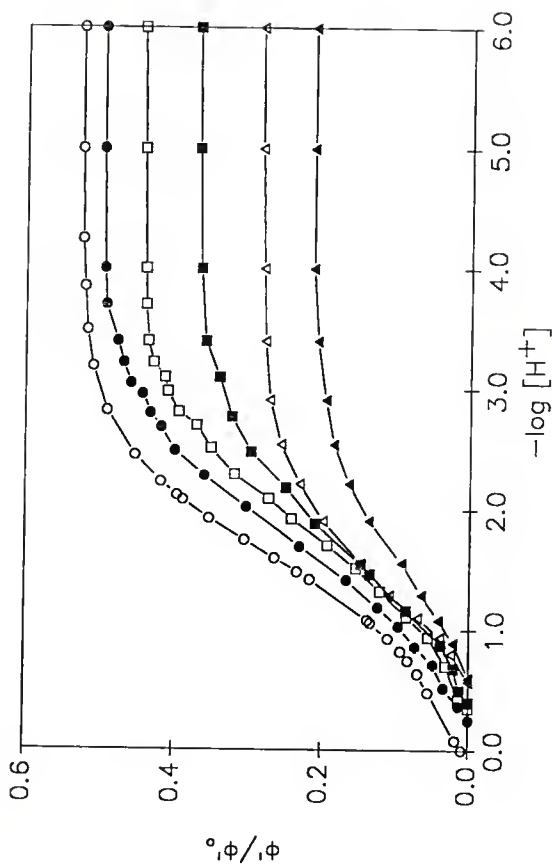


Figure 3.38. Variation of the relative quantum yields of fluorescence of the conjugate base (ϕ/ϕ_0) species of 2-hydroxybiphenyl with the formal hydrogen ion concentration $[H^+]$ in aqueous ethanol.
 -○- ($X_e=0.000$), -●- ($X_e=0.033$), -□- ($X_e=0.072$),
 -■- ($X_e=0.117$), -△- ($X_e=0.236$), -▲- ($X_e=0.317$)

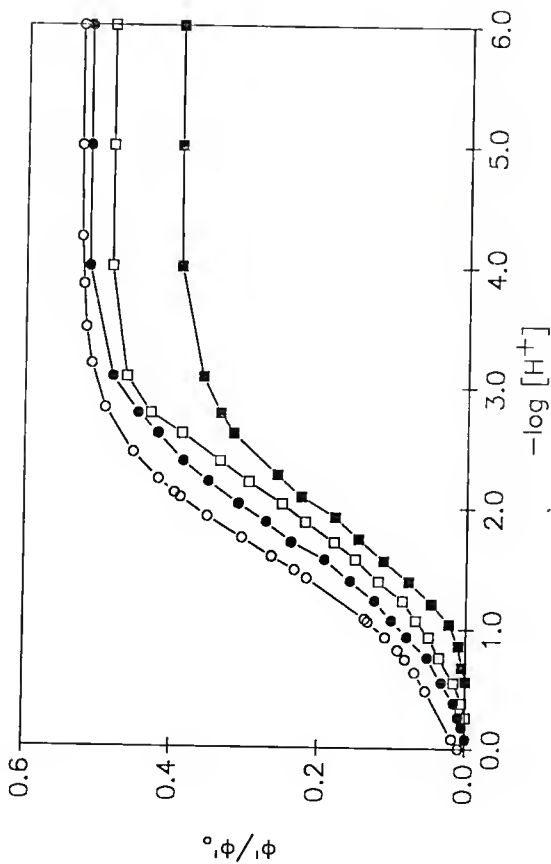


Figure 3.39. Variation of the relative quantum yields of fluorescence of the conjugate base (ϕ'/ϕ) species of 2-hydroxybiphenyl with the formal hydrogen ion concentration $[H^+]$ in aqueous isopropanol. -O- ($X_1=0.000$), -●- ($X_1=0.026$), -□- ($X_1=0.057$), -■- ($X_1=0.094$)

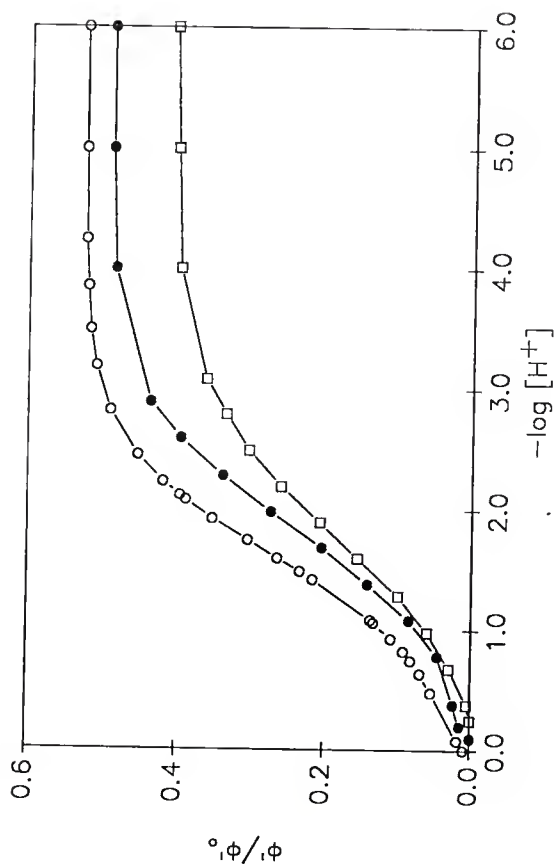


Figure 3.40. Variation of the relative quantum yields of fluorescence of the conjugate base (ϕ'/ϕ) species of 2-hydroxybiphenyl with the formal hydrogen ion concentration $[H^+]$ in aqueous t-butanol. —○— ($X_t=0.000$), —●— ($X_t=0.021$), —□— ($X_t=0.046$)

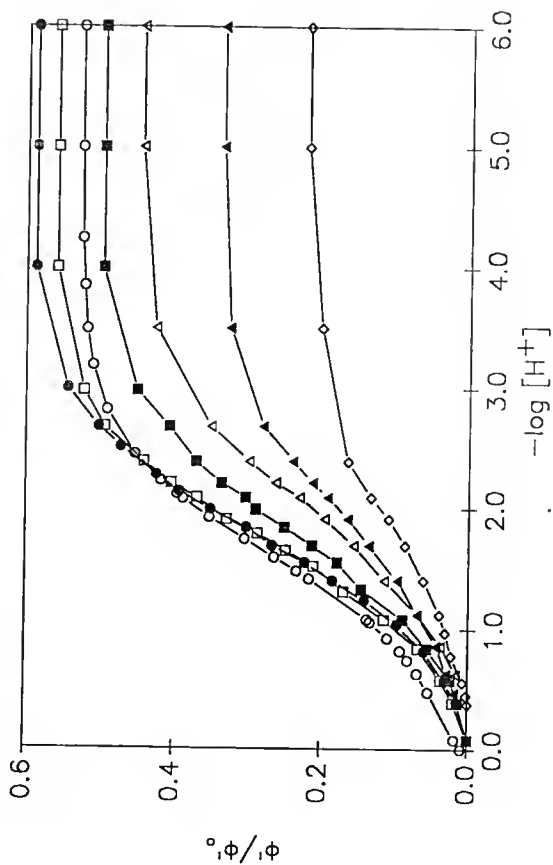


Figure 3.41. Variation of the relative quantum yields of fluorescence of the conjugate base (ϕ/ϕ_0) species of 2-hydroxybiphenyl with the formal hydrogen ion concentration $[H^+]$ in aqueous acetonitrile. —○— ($X_a=0.000$), —●— ($X_a=0.037$), —◻— ($X_a=0.079$), —◼— ($X_a=0.128$), —▽— ($X_a=0.186$), —△— ($X_a=0.256$), —◇— ($X_a=0.340$), —◄— ($X_a=0.445$)

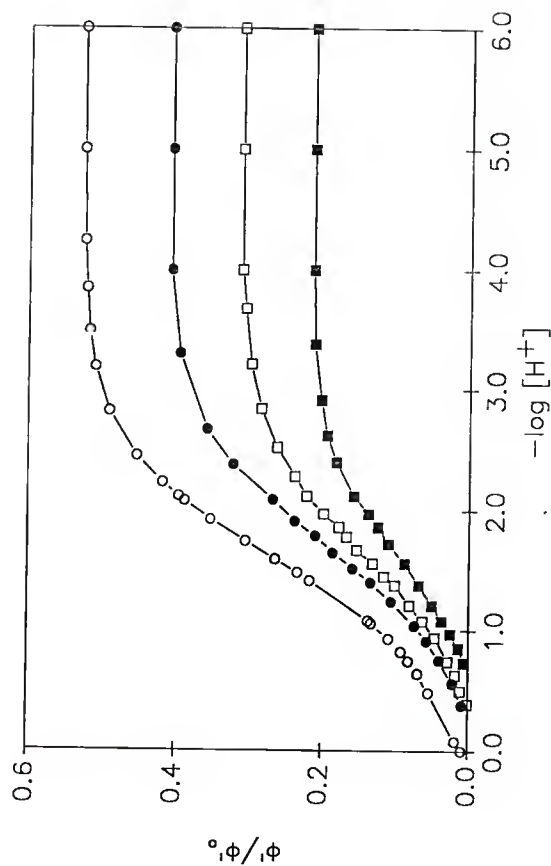


Figure 3.42. Variation of the relative quantum yields of fluorescence of the conjugate base (ϕ'/ϕ) species of 2-hydroxybiphenyl with the formal hydrogen ion concentration $[H^+]$ in aqueous dioxane. -O- ($X_d=0.000$), -●- ($X_d=0.021$), -□- ($X_d=0.046$), -■- ($X_d=0.083$)

relative quantum yields of the neutral molecule, ϕ/ϕ_0 , and of the anion, ϕ'/ϕ'_0 , are invariant with respect to $[H^+]$ and $[OH^-]$ and are related to the kinetics of dissociation in the excited state in mixed aqueous organic solvents as well as in water by (74,75,141):

$$\frac{\phi'/\phi'_0}{\phi/\phi_0} = k_a \tau_0 \quad (1.18)$$

where k_a is the rate constant for prototropic dissociation of 2-hydroxybiphenyl in the excited state, and τ_0 is the lifetime of neutral species in the absence of proton transfer. It was previously shown for some hydroxyaromatics (5,9,74,75) and now above for 2-hydroxybiphenyl, that $(\phi'/\phi'_0)/(\phi/\phi_0)$ and therefore $k_a \tau_0$ varies exponentially with the mole fraction of organic cosolvent and that the slope of the plot of $\log k_a \tau_0$ against X_c (the mole fraction of cosolvent) is a function of the activity of water and the distance separating the fully hydrated hydrogen ion and the fully hydrated conjugate base in the encounter complex (or solvent separated ion-pair) (75).

At $[H^+] \geq 1 \times 10^{-3} M$, the fluorescence of the conjugate base decreased and eventually disappeared concomitant with the increase in fluorescence from the neutral molecule. This behavior is typical of a hydroxyaromatic compound and

corresponds to reprotonation of the excited conjugate base subsequent to dissociation of the neutral molecule (141). At $[H^+] < 1 \times 10^{-3} \text{ M}$, the kinetics of reprotonation are such that there is not enough $[H^+]$ present to reprotonate the 2-hydroxybiphenylate anion that can be measured, during the lifetime of its excited state, τ'_0 . In the acidity region where ϕ/ϕ_0 and ϕ'/ϕ'_0 vary with $[H^+]$, the relationship between them is given by (74,141):

$$\frac{\phi/\phi_0}{\phi'/\phi'_0} = \frac{1}{K_a \tau_0} + \frac{k_b \tau'_0}{K_a \tau_0} F[H^+] \quad (1.17)$$

where k_b is the rate constant for reprotonation of 2-hydroxybiphenylate (ArO^-) in the excited state and F is the Bronsted kinetic activity factor (143,144) which corrects each experimental value of k_b to zero ionic strength in the solvent mixture chosen. F is derived along the same lines as the extended Debye-Hückel equation (187) for the reaction:



where X is the transition state (or activated complex) preceding the formation of ArOH , and is given for a univalent ionic reaction such as the hydrogen ion-hydroxybiphenylate reaction by:

$$-\log F = \frac{2A\sqrt{\mu}}{1 + aB\sqrt{\mu}} \quad (3.26)$$

where μ is the ionic strength. A is related to the dielectric constant, ϵ , (188) and the absolute temperature, T , by (189,190).

$$A = 1.826 \times 10^6 (\epsilon T)^{-3/2}$$

B is related to ϵ and T by

$$B = 5.031 \times 10^9 (\epsilon T)^{-1/2}$$

Finally, a is the mean distance of closest approach of the fully solvated proton and the 2-hydroxybiphenylate anion. The values of a are listed in Table 3.10, above.

Values of ϵ , A and B for various organic cosolvent water mixtures at 25°C in which fluorimetric titrations were carried out are listed in tables 3.19-3.24. As seen in figures 3.43-3.48, plots of $(\phi/\phi_o)/(\phi'/\phi'_o)$ versus $F[H^+]$ are linear up to at least $[H^+] = 0.01$ M and often up to 0.1 M. Moreover, the ordinate intercepts of these lines, which according to equation (3.26) should correspond to $(k_a\tau_o)^{-1}$, agree well with the corresponding terms calculated from equation (1.18). Although $k_a\tau_o$ is extremely solvent

Table 3.19.

Debye-Hückel terms (A and B) at different dielectric strengths (ϵ) in methanol-water solutions along with the products of the rate constants and corresponding fluorescence decay-times $k_a\tau_o$, calculated from equation (1.18) and the reciprocal of the intercept of equation (1.17) and $k_b\tau_o$, determined from the product of $k_a\tau_o$ and the slope of equation (1.17).

ϕ_m	X_m	ϵ	A	$B \times 10^{-7}$	$k_a\tau_o^a$	$k_a\tau_o^b$	$k_b\tau_o^a$	$k_b\tau_o^b$
0	0.000	78.54	0.509	3.29	1.11 ± 0.01	0.81 ± 0.19	118 ± 3	86 ± 20
10	0.047	75.01	0.546	3.36	1.00 ± 0.03	0.84 ± 0.27	152 ± 5	128 ± 41
20	0.100	70.94	0.594	3.46	1.01 ± 0.03	0.92 ± 0.09	136 ± 4	124 ± 12
30	0.161	66.69	0.652	3.57	0.86 ± 0.03	0.82 ± 0.09	122 ± 4	118 ± 13
40	0.229	62.24	0.723	3.69	0.69 ± 0.06	0.63 ± 0.08	116 ± 10	105 ± 13
50	0.308	57.87	0.806	3.83	0.45 ± 0.06	0.43 ± 0.04	114 ± 15	108 ± 10
60	0.401	52.83	0.924	4.01	0.25 ± 0.06	0.24 ± 0.02	119 ± 29	113 ± 9
70	0.510	47.68	1.078	4.22	0.20 ± 0.06	0.20 ± 0.01	104 ± 31	105 ± 5

a) calculated from equation (1.18)

b) calculated from equation (1.17)

Table 3.20.

Debye-Hückel terms (A and B) at different dielectric strengths (ϵ) in ethanol-water solutions along with the products of the rate constants and corresponding fluorescence decay-times $k_a\tau_o$, calculated from equation (1.18) and the reciprocal of the intercept of equation (1.17) and $k_b\tau_o$, determined from the product of $k_a\tau_o$ and the slope of equation (1.17).

ϕ_e	X_e	ϵ	A	$B \times 10^{-7}$	$k_a\tau_o^a$	$k_a\tau_o^b$	$k_b\tau_o^a$	$k_b\tau_o^b$
0	0.000	78.54	0.509	3.29	1.11 ± 0.01	0.81 ± 0.19	118 ± 3	86 ± 20
10	0.033	74.32	0.554	3.38	1.10 ± 0.03	0.85 ± 0.23	156 ± 4	120 ± 32
20	0.072	69.33	0.615	3.50	1.02 ± 0.03	0.94 ± 0.23	136 ± 4	125 ± 31
30	0.117	64.04	0.693	3.64	0.82 ± 0.03	0.80 ± 0.19	136 ± 5	133 ± 32
40	0.171	58.60	0.791	3.80	0.58 ± 0.03	0.59 ± 0.13	105 ± 5	107 ± 24
50	0.236	52.83	0.924	4.01	0.40 ± 0.03	0.43 ± 0.14	93 ± 7	100 ± 33
60	0.317	33.12	1.111	4.26	0.27 ± 0.03	0.28 ± 0.05	122 ± 14	126 ± 22

a) calculated from equation (1.18)

b) calculated from equation (1.17)

Table 3.21.

Debye-Hückel terms (A and B) at different dielectric strengths (ϵ) in isopropanol-water solutions along with the products of the rate constants and corresponding fluorescence decay-times $k_a\tau_o$, calculated from equation (1.18) and the reciprocal of the intercept of equation (1.17) and $k_b\tau_o$, determined from the product of $k_a\tau_o$ and the slope of equation (1.17).

ϕ_1	X_1	ϵ	A	$B \times 10^{-7}$	$k_a\tau_o^a$	$k_a\tau_o^b$	$k_b\tau_o^a$	$k_b\tau_o^b$
0	0.000	78.54	0.509	3.29	1.11 ± 0.01	0.81 ± 0.19	118 ± 3	80 ± 20
10	0.026	74.11	0.556	3.38	1.06 ± 0.05	0.90 ± 0.23	144 ± 7	123 ± 23
20	0.057	68.82	0.622	3.51	0.93 ± 0.05	0.73 ± 0.22	139 ± 7	110 ± 33
30	0.094	62.51	0.718	3.68	0.64 ± 0.05	0.63 ± 0.11	110 ± 9	108 ± 11

a) calculated from equation (1.18)

b) calculated from equation (1.17)

Table 3.22.

Debye-Hückel terms (A and B) at different dielectric strengths (ϵ) in t-butanol-water solutions along with the products of the rate constants and corresponding fluorescence decay-times $k_a\tau_o$, calculated from equation (1.18) and the reciprocal of the intercept of equation (1.17) and $k_b\tau_o$, determined from the product of $k_a\tau_o$ and the slope of equation (1.17).

$\%t$	X_t	ϵ	A	$B \times 10^{-7}$	$k_a\tau_o^a$	$k_a\tau_o^b$	$k_b\tau_o^a$	$k_b\tau_o^b$
0	0.000	78.54	0.509	3.29	1.11 ± 0.01	0.81 ± 0.19	118 ± 3	80 ± 20
10	0.021	73.40	0.564	3.40	0.94 ± 0.03	0.81 ± 0.21	141 ± 4	121 ± 31
20	0.046	67.27	0.643	3.55	0.66 ± 0.03	0.66 ± 0.03	113 ± 5	113 ± 5

a) calculated from equation (1.18)

b) calculated from equation (1.17)

Table 3.23.

Debye-Hückel terms (A and B) at different dielectric strengths (ϵ) in acetonitrile-water solutions along with the products of the rate constants and corresponding fluorescence decay-times $k_a\tau_o$, calculated from equation (1.18) and the reciprocal of the intercept of equation (1.17) and $k_b\tau_o'$, determined from the product of $k_a\tau_o$ and the slope of equation (1.17).

ϕ_a	X_a	ϵ	A	$B \times 10^{-7}$	$k_a\tau_o^a$	$k_a\tau_o^b$	$k_b\tau_o'^a$	$k_b\tau_o'^b$
0	0.000	78.54	0.509	3.29	1.11 ± 0.01	0.81 ± 0.19	118 ± 3	86 ± 20
10	0.037	75.70	0.539	3.35	1.30 ± 0.02	1.13 ± 0.15	146 ± 2	126 ± 17
20	0.079	72.24	0.578	3.43	1.43 ± 0.02	1.31 ± 0.11	125 ± 2	114 ± 10
30	0.128	67.85	0.635	3.54	1.27 ± 0.02	1.24 ± 0.33	112 ± 2	109 ± 29
40	0.186	63.25	0.706	3.66	0.99 ± 0.02	0.96 ± 0.44	137 ± 3	132 ± 61
50	0.256	58.47	0.794	3.8x	0.80 ± 0.02	0.70 ± 0.43	152 ± 4	133 ± 82
60	0.340	53.78	0.900	3.97	0.51 ± 0.02	0.47 ± 0.34	127 ± 5	117 ± 85
70	0.445	49.13	1.031	4.15	0.28 ± 0.02	0.26 $\pm 0.8x$	132 ± 9	122 ± 122

a) calculated from equation (1.18)

b) calculated from equation (1.17)

Table 3.24.

Debye-Hückel terms (A and B) at different dielectric strengths (ϵ) in dioxane-water solutions along with the products of the rate constants and corresponding fluorescence decay-times $k_1\tau_0$, calculated from equation (1.18) and the reciprocal of the intercept of equation (1.17) and $k_2\tau_0$, determined from the product of $k_1\tau_0$ and the slope of equation (1.17).

Φ_d	X_d	ϵ	A	$B \times 10^{-7}$	$k_2\tau_0^a$	$k_2\tau_0^b$	$k_2\tau_0^{1/a}$	$k_2\tau_0^{1/b}$
0	0.000	78.54	0.509	3.29	1.11 ± 0.01	0.81 ± 0.19	118 ± 3	80 ± 20
10	0.023	69.87	0.608	3.48	0.69 ± 0.05	0.67 ± 0.22	120 ± 9	117 ± 38
20	0.050	60.62	0.752	3.74	0.45 ± 0.05	0.46 ± 0.08	103 ± 11	105 ± 18
30	0.083	51.74	0.954	4.05	0.27 ± 0.05	0.29 ± 0.01	106 ± 20	114 ± 4

a) calculated from equation (1.18)

b) calculated from equation (1.17)

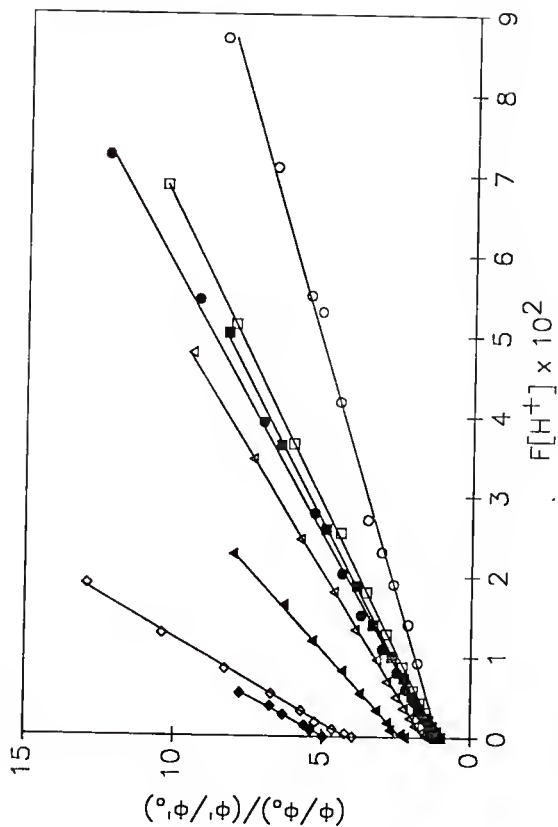


Figure 3.43. Plots of $(\phi/\phi_0)/(\phi'/\phi'_0)$ versus $F[H^+]$ for 2-hydroxybiphenyl in aqueous methanol, where the mole fraction of methanol (X_m) is
 -○- ($X_m=0.000$), -●- ($X_m=0.047$), -□- ($X_m=0.100$), -■- ($X_m=0.161$),
 -▽- ($X_m=0.229$), -▲- ($X_m=0.308$), -◇- ($X_m=0.401$), -◆- ($X_m=0.510$).

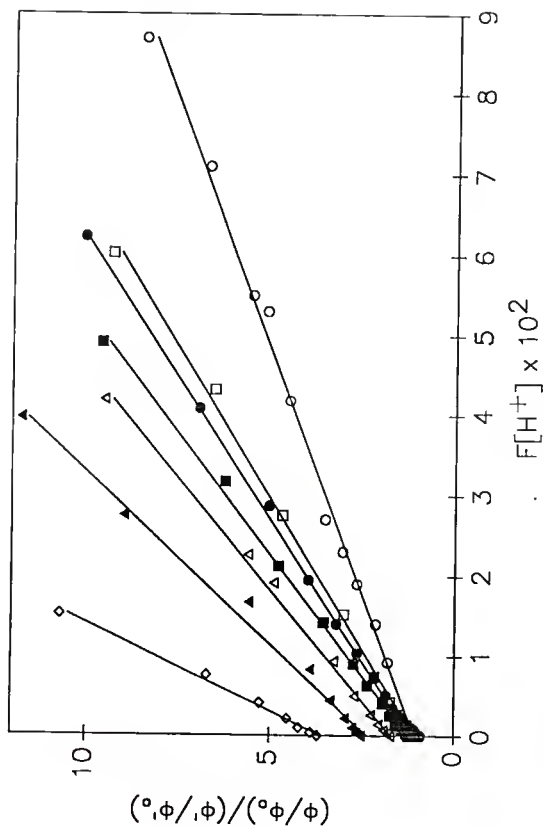


Figure 3.44. Plots of $(\phi/\phi_0)/(\phi'/\phi'_0)$ versus $F[H^+]$ for 2-hydroxybiphenyl in aqueous ethanol, where the mole fraction of ethanol (X_e) is
 -○- ($X_e=0.000$), -●- ($X_e=0.033$), -□- ($X_e=0.072$), -■- ($X_e=0.117$),
 -▽- ($X_e=0.171$), -▲- ($X_e=0.236$), -◇- ($X_e=0.317$).

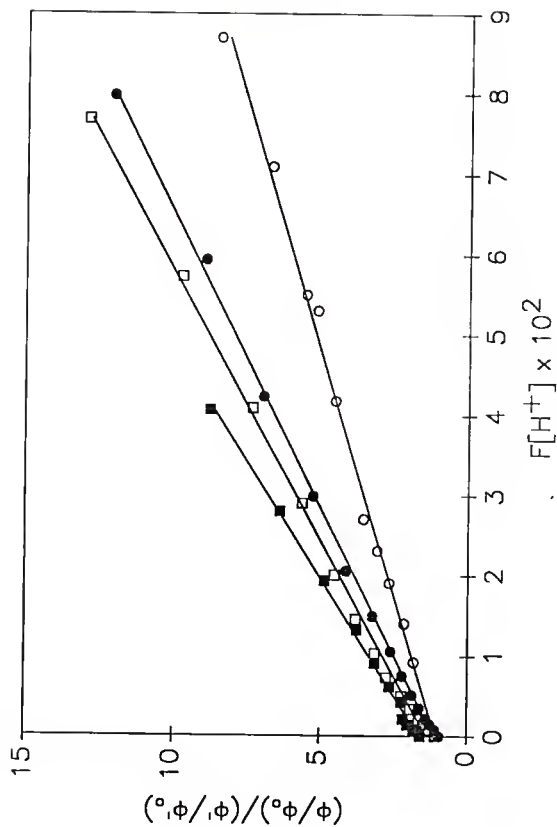


Figure 3.45. Plots of $(\phi/\phi_0)/(\phi'/\phi'_0)$ versus $F[H^+]$ for 2-hydroxybiphenyl in aqueous isopropanol, where the mole fraction of isopropanol (X_1) is -○- ($X_1=0.000$), -●- ($X_1=0.026$), -□- ($X_1=0.057$), -■- ($X_1=0.194$).

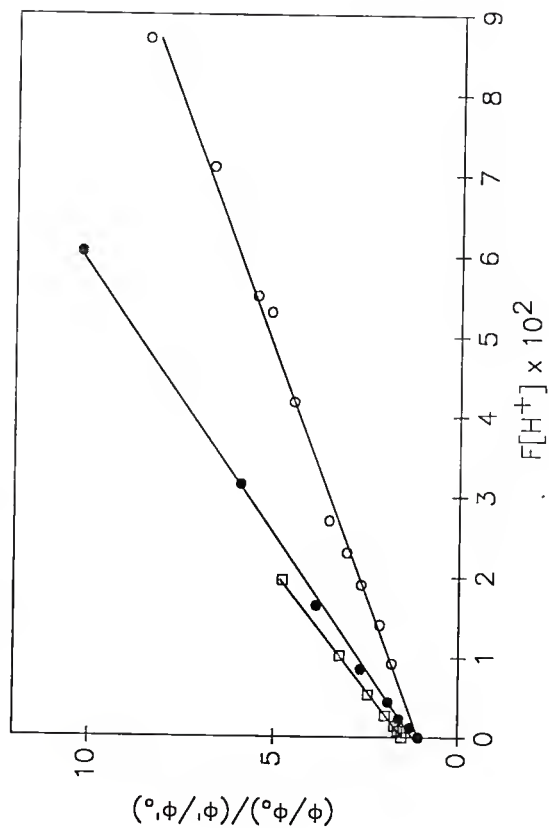


Figure 3.46. Plots of $(\phi/\phi_0)/(\phi'/\phi'_0)$ versus $F[H^+]$ for 2-hydroxybiphenyl in aqueous t-butanol, where the mole fraction of t-butanol (X_t) is -O- ($X_t=0.000$), -●- ($X_t=0.026$), -□- ($X_t=0.046$).

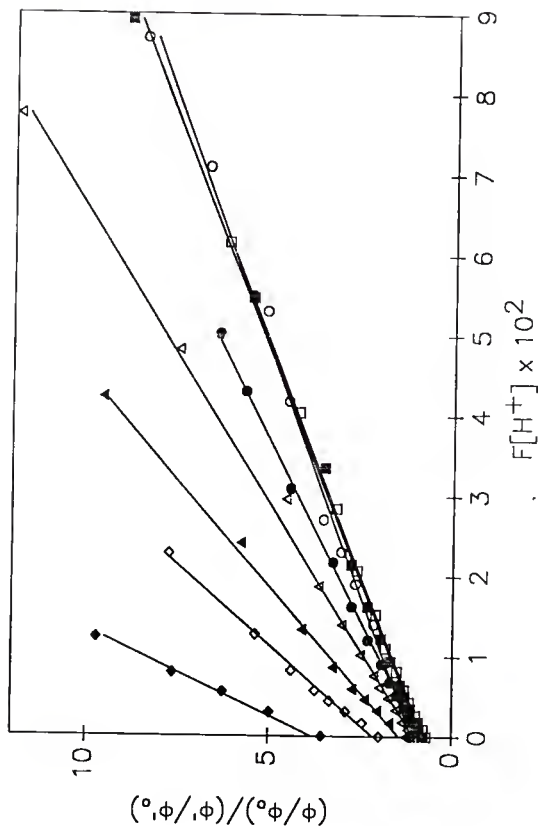


Figure 3.47. Plots of $(\phi/\phi_0)/(\phi'/\phi'_0)$ versus $F[H^+]$ for 2-hydroxybiphenyl in aqueous acetonitrile, where the mole fraction of acetonitrile (X_a) is -○- ($X_a=0.000$), -●- ($X_a=0.037$), -□- ($X_a=0.079$), -■- ($X_a=0.128$), -▽- ($X_a=0.186$), -▲- ($X_a=0.256$), -◇- ($X_a=0.340$), -◆- ($X_a=0.445$).

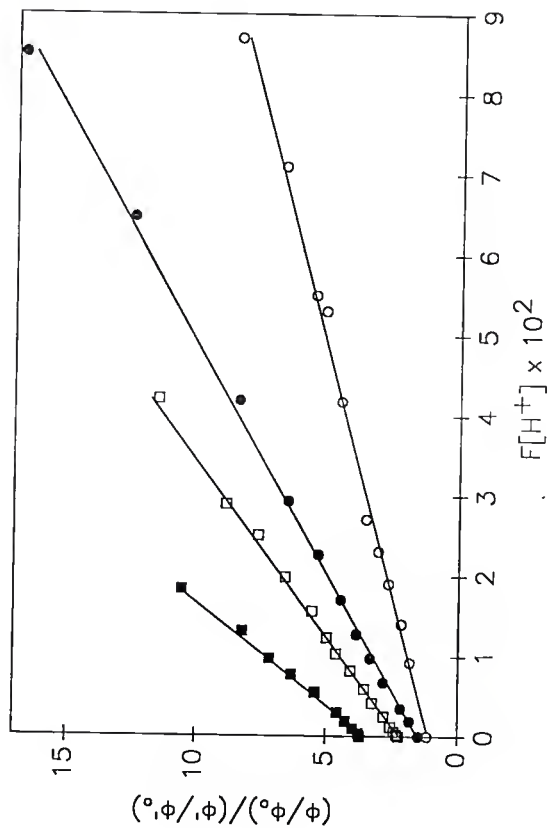


Figure 3.48. Plots of $(\phi/\phi_0)/(\phi'/\phi'_0)$ versus $F[H^+]$ for 2-hydroxybiphenyl in aqueous dioxane, where the mole fraction of dioxane (X_d) is $-O-$ ($X_d=0.000$), $-●-$ ($X_d=0.023$), $-\square-$ ($X_d=0.050$), $-\blacksquare-$ ($X_d=0.083$).

dependent, $k_b r'_o$ (tables 3.19-3.24) is virtually independent of the mole fraction of the solvent.

The linearities of the plots of $(\phi/\phi_o)/(\phi'/\phi'_o)$ versus $F[H^*]$ and the excellent agreement between the values of $k_a r_o$ obtained from these least-squares plots, which are largely largely determined by the pH dependence of the back reaction, and those obtained from equation (1.18), which are calculated independent of any assumptions regarding pH, lead to the conclusion that the form of the Brønsted kinetic activity factor chosen here is a reasonably accurate means of correcting the parameters of the chosen reaction for nonideal behavior. Furthermore, it appears that, while the solvent influence on the dissociation reaction cannot be described by continuum electrostatics (9), the influence of the solvent on the reprotonation reaction can. Accordingly, since F is a good activity factor for the overall reprotonation reaction, it was assumed that $f_{H^+} = \sqrt{F}$ should be a good activity coefficient for the solvated proton. Then, in any of the mixed solvent systems, the activity of the hydrogen ion is:

$$a_{H^+} = f_{H^+}[H^*] \quad (3.27)$$

and the pH is:

$$pH = -\log a_{H^+} = \frac{1.826 \times 10^6 (\epsilon T)^{-\frac{3}{2}} \sqrt{\mu}}{1 + 5.03 \times 10^9 a \sqrt{\frac{\mu}{\epsilon T}}} - \log[H^+] \quad (3.28)$$

The pH of dilute sulfuric acid solutions for several aqueous-organic mixtures at 25°C as a function of the reciprocal hydrogen ion formal concentration ($-\log[H^+]$) and the mole fraction of organic cosolvent are given in Tables 3.25-3.30. According to Tables 3.25-3.30, the influence of the cosolvent on the pH in these systems is remarkably small suggesting that H^+ is solvated exclusively by water. Such results tend to provide support for the transition state (i.e., hydration) approach to proton transfer in mixed solvents that was utilized to probe water structure and its activity above.

The approach described here ought to be applicable to any aqueous-organic solvent system in which excited state proton transfer of the kind demonstrated here is observed.

Table 3.26. pH of dilute sulfuric acid solutions in aqueous ethanol as a function of the logarithm of the reciprocal hydrogen ion formal concentration ($-\log[H^+]$) and the mole fraction of ethanol (X_e).

$-\log[H^+]$	pH						
	$X_e=0$	$X_e=0.033$	$X_e=0.072$	$X_e=0.117$	$X_e=0.171$	$X_e=0.236$	$X_e=0.317$
3.40	3.41	3.41	3.42	3.43	3.43	3.43	3.43
3.20	3.21	3.22	3.22	3.22	3.23	3.23	3.24
3.00	3.02	3.02	3.02	3.03	3.03	3.04	3.04
2.90	2.92	2.92	2.93	2.93	2.93	2.94	2.95
2.80	2.82	2.83	2.83	2.83	2.84	2.85	2.85
2.70	2.72	2.73	2.73	2.74	2.74	2.75	2.75
2.60	2.62	2.63	2.63	2.64	2.64	2.65	2.66
2.50	2.53	2.53	2.54	2.54	2.55	2.56	2.56
2.40	2.43	2.44	2.44	2.45	2.45	2.46	2.48
2.30	2.33	2.34	2.34	2.35	2.36	2.37	2.38
2.20	2.23	2.24	2.25	2.25	2.26	2.28	2.29
2.10	2.13	2.15	2.15	2.16	2.16	2.18	2.20
2.00	2.04	2.05	2.05	2.06	2.07	2.09	2.10
1.90	1.95	1.95	1.96	1.96	1.97	1.99	2.01
1.80	1.85	1.86	1.86	1.87	1.88	1.90	1.92
1.70	1.75	1.76	1.76	1.77	1.78	1.81	1.83
1.60	1.66	1.66	1.67	1.68	1.69	1.71	1.73
1.50	1.57	1.57	1.57	1.58	1.59	1.62	1.64
1.40	1.47	1.47	1.48	1.49	1.50	1.53	
1.30	1.38	1.37	1.38	1.39	1.40	1.43	
1.20	1.28	1.28	1.29	1.29	1.31	1.34	
1.10	1.19	1.18	1.19	1.20	1.21		
1.00	1.10	1.09	1.09				
0.90	1.00	0.99	0.99				

Table 3.27. pH of dilute sulfuric acid solution in aqueous isopropanol as a function of the logarithm of the reciprocal hydrogen ion formal concentration ($-\log[H^+]$) and the mole fraction of isopropanol (X_i).

$-\log[H^+]$	pH		
	$X_i=0$	$X_i=0.026$	$X_i=0.057$
3.40	3.41	3.41	3.42
3.20	3.21	3.21	3.22
3.00	3.02	3.02	3.03
2.90	2.92	2.92	2.93
2.80	2.82	2.83	2.83
2.70	2.72	2.73	2.73
2.60	2.62	2.63	2.63
2.50	2.53	2.53	2.54
2.40	2.43	2.44	2.44
2.30	2.33	2.34	2.34
2.20	2.23	2.24	2.25
2.10	2.13	2.14	2.15
2.00	2.04	2.05	2.05
1.90	1.95	1.95	1.96
1.80	1.85	1.85	1.86
1.70	1.75	1.76	1.77
1.60	1.66	1.66	1.67
1.50	1.57	1.56	1.57
1.40	1.47	1.47	1.47
1.30	1.38	1.37	1.38
1.20	1.28	1.27	1.28
1.10	1.19	1.18	1.18
1.00	1.10	1.08	1.09

Table 3.28.

pH of dilute sulfuric acid solution in aqueous t-butanol as a function of the logarithm of the reciprocal hydrogen ion formal concentration ($-\log[H^+]$) and the mole fraction of t-butanol (X_t).

$-\log[H^+]$	pH		
	$X_t=0$	$X_t=0.021$	$X_t=0.046$
3.40	3.41	3.41	3.42
3.20	3.21	3.22	3.22
3.00	3.02	3.02	3.02
2.90	2.92	2.92	2.93
2.80	2.82	2.82	2.83
2.70	2.72	2.73	2.73
2.60	2.62	2.63	2.63
2.50	2.53	2.53	2.54
2.40	2.43	2.43	2.44
2.30	2.33	2.34	2.34
2.20	2.23	2.24	2.24
2.10	2.13	2.14	2.15
2.00	2.04	2.04	2.05
1.90	1.95	1.95	1.95
1.80	1.85	1.85	1.80
1.70	1.75	1.75	
1.60	1.66	1.65	
1.50	1.57	1.56	
1.40	1.47	1.46	
1.30	1.38	1.36	
1.20	1.28	1.27	
1.10	1.19	1.17	
1.00	1.10		

Table 3.29.

pH of dilute sulfuric acid solutions in aqueous acetonitrile as a function of the logarithm of the reciprocal hydrogen ion formal concentration ($-\log[H^+]$) and the mole fraction of acetonitrile (X_a).

$-\log[H^+]$	pH									
	$X_a=0$	$X_a=0.037$	$X_a=0.079$	$X_a=0.428$	$X_a=0.186$	$X_a=0.256$	$X_a=0.340$	$X_a=0.445$		
3.00	3.02	3.02	3.02	3.02	3.03	3.03	3.04	3.04	3.04	3.04
2.90	2.92	2.92	2.92	2.93	2.93	2.94	2.94	2.94	2.94	2.94
2.80	2.82	2.83	2.83	2.83	2.83	2.84	2.84	2.84	2.84	2.84
2.70	2.72	2.73	2.73	2.73	2.74	2.74	2.75	2.75	2.75	2.75
2.60	2.62	2.63	2.63	2.64	2.64	2.65	2.65	2.65	2.65	2.65
2.50	2.53	2.53	2.54	2.54	2.55	2.55	2.56	2.56	2.56	2.56
2.40	2.43	2.44	2.44	2.44	2.45	2.46	2.46	2.47	2.47	2.47
2.30	2.33	2.34	2.34	2.35	2.35	2.36	2.37	2.37	2.37	2.37
2.20	2.23	2.24	2.24	2.25	2.26	2.26	2.27	2.27	2.28	2.28
2.10	2.13	2.14	2.15	2.15	2.16	2.17	2.18	2.18	2.15	2.15
2.00	2.04	2.05	2.05	2.06	2.07	2.07	2.05	2.05	2.05	2.05
1.90	1.95	1.95	1.95	1.96	1.97	1.98	1.96	1.96	1.96	1.96
1.80	1.85	1.85	1.86	1.86	1.88	1.88	1.86	1.86	1.86	1.86
1.70	1.75	1.76	1.76	1.77	1.77	1.78	1.78	1.78	1.76	1.76
1.60	1.66	1.66	1.66	1.67	1.67	1.70	1.67	1.67	1.67	1.67
1.50	1.57	1.56	1.57	1.57	1.57	1.60	1.57	1.57	1.57	1.57
1.40	1.47	1.47	1.47	1.48	1.48	1.51	1.48	1.48	1.48	1.48
1.30	1.38	1.37	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38
1.20	1.28	1.28	1.28	1.28	1.29	1.29	1.29	1.29	1.29	1.29
1.10	1.19	1.19	1.18	1.20	1.20	1.20	1.20	1.20	1.20	1.20
1.00	1.10	1.08	1.09							

Table 3.30. pH of dilute sulfuric acid solutions in aqueous dioxane as a function of the logarithm of the reciprocal hydrogen ion formal concentration ($-\log[H^+]$) and the mole fraction of dioxane (X_d).

$\log[H^+]$	pH			
	$X_d=0$	$X_d=0.023$	$X_d=0.050$	$X_d=0.083$
3.40	3.41	3.42	3.42	3.42
3.20	3.21	3.22	3.22	3.23
3.00	3.02	3.02	3.03	3.03
2.90	2.92	2.92	2.93	2.94
2.80	2.82	2.83	2.83	2.84
2.70	2.72	2.73	2.74	2.74
2.60	2.62	2.63	2.64	2.65
2.50	2.53	2.53	2.54	2.55
2.40	2.43	2.44	2.45	2.46
2.30	2.33	2.34	2.35	2.36
2.20	2.23	2.24	2.25	2.26
2.10	2.13	2.15	2.16	2.17
2.00	2.04	2.05	2.06	2.07
1.90	1.95	1.95	1.96	1.98
1.80	1.85	1.85	1.86	1.88
1.70	1.75	1.76	1.77	1.78
1.60	1.66	1.66	1.67	1.69
1.50	1.57	1.56	1.57	1.59
1.40	1.47	1.47	1.48	1.50
1.30	1.38	1.37	1.38	
1.20	1.29	1.27	1.29	
1.10	1.19	1.17		
1.00	1.10			

CHAPTER 4 CONCLUSIONS

The lowest excited singlet state properties of 6-methoxyquinoline and 3 isomeric hydroxybiphenyls have been investigated in aqueous media with a combination of steady-state and time-resolved fluorescence techniques.

Direct evidence of biexponential decay profiles from the neutral species of 6-methoxyquinoline in the pH range 11.5-12.5 has helped to resolve the discrepancies that existed in the literature regarding its excited state kinetic properties in aqueous media. It has now been shown in agreement with Pines et al. (43) that the dissociation constant of 6-methoxyquinoline in the lowest excited singlet state (pK_a') is 11.84 ± 0.03 and not 11.4 as originally reported (152).

It has been found that the 3 isomeric hydroxybiphenyls possess excited state activity in support of observations made by Bridges et al. (156), with one exception. They reported that the 4-hydroxybiphenyl did not exhibit prototropic dissociation in the lowest excited singlet state. This discrepancy was explained by the fact that the primary analyte used by Bridges et al. contained 5% (v/v)

ethanol which has been shown in the mixed aqueous organic portion of this study to be an inhibitor of dissociation in the lowest excited singlet state. Further, evidence has been provided to show that the magnitudes of the dissociation rate constants in the lowest excited singlet state can be rationalized in terms of the vectorial coupling of the $L_b \leftarrow$ ¹A transition moment of the benzene ring to which the hydroxy group is bonded, with the moment corresponding to the direction of electronic interaction of the benzene substituent with the ring.

With the aqueous properties of 6-methoxyquinoline and the hydroxybiphenyls fully established, it has been demonstrated that excited state proton transfer in partially aqueous solutions can be used to characterize water structure and determine the number of water molecules that participate in the reaction at any given solvent composition. It is evident that the dissociation reaction of directly excited 2-hydroxybiphenyl and the hydrolysis reaction of directly excited 6-methoxyquinoline is strongly solvent dependent and independent of solution acidity and/or basicity. In aqueous-organic solutions, the rate constant of dissociation (or hydrolysis) decreases biexponentially with increasing mole fraction of organic cosolvent and eventually is completely inhibited. The mole fraction range at which the inhibition of proton transfer takes place is dependent

upon which cosolvent has been used. It has been found that the slope of the logarithm of the dissociation (or hydrolysis) rate constant against the mole fraction of organic cosolvent correlates well with the ratio of the diffusion volume of excited 2-hydroxybiphenyl (or 6-methoxyquinoline) to the molecular volume of the organic cosolvent. In fact, both probes are affected to the same degree. This provides support for transition state (or hydration) approach to proton transfer in mixed solvents initially used by Kelly (151). From the slope of the plot of the logarithm of the dissociation (or hydrolysis) rate constant against the logarithm of the water activity, evidence has been put forth to show that excited state proton transfer provides a means to determine how many free water molecules participate in the proton transfer process in mixed aqueous-organic solutions, presumably, in the form of water clusters.

Interestingly enough, it was suggested by Kelly that in mixed solvents a region of water activity should be reached in which small clusters of water become the predominant proton accepting species. Further, he speculated that if the size of the water cluster changes then so should the slopes of the $\log(k)$ vs $\log(a_w)$ plots. These concepts have been verified. For example, it has been found that water becomes increasingly more structured as the size and bulk of the

organic cosolvent is increased. In the alcohol series, methanol rigidifies and/or structures water to a lesser degree than ethanol, isopropanol and t-butanol, in that order. Thus, the clusters of water initially formed in methanol are much smaller than those initially formed in t-butanol. However as the alcohol concentration is increased, water clusters break down. In an attempt to achieve a thermodynamically favored configuration, new cluster of water are formed. From the data given above, it appears that in most solvent systems, this cluster is comprised of exactly five molecules of water, i.e., the primary pentagonal unit. The fact that the original clusters are divisible by five, tends to suggest that the primary pentagonal water unit is the primary proton accepting species. It is from this unit that the H_3O_4^+ ion must evolve. However, because the available data on water activity in mixed solvents is at best questionable, additional work in this area is needed.

Finally, excited state proton transfer can be used to establish nearly accurate and thermodynamically significant pH in aqueous-organic solvent systems. From the fluorescence of 2-hydroxybiphenyl, the reprotonation rate constant was found to depend predominantly on the acidity of the solution and only on the continuum properties of the solvent. The linear relationship between the ratio of the relative

fluorescence efficiencies of 2-hydroxybiphenyl and its conjugate base and hydrogen ion concentration were obtained only when proper Bronsted activity factors were included in the relationship. These factors are related to the proton activity coefficient, which in itself is a complex function of the bulk dielectric constant and temperature of the solution, by a simple quadratic expression (see equation (3.28)). As can be seen in Tables 3.25-3.30, using this approximation has lead to the calculation of pH in aqueous-organic solutions containing mole fractions of methanol, ethanol, isopropanol, t-butanol, acetonitrile and dioxane up to 0.5, 0.3, 0.1, 0.05, 0.5 and 0.1, respectively.

The approach here ought to be applicable to any aqueous-organic solvent system in which excited state proton transfer of the kind demonstrated here is observed.

REFERENCES

1. Urban, W., and Weller, A., Ber. Bunsenges, Physick Chem., 67 (1963), 787.
2. Trieff, N.M., and Sundheim, B.R., J. Phys. Chem., 69 (1965), 2044.
3. Weller, A., in Progress in Reaction Kinetics, I, ed. G. Porter (Pergamon Press, New York, 1961, p. 1-45).
4. Williamson, B., and La Mer, V.K., J. Am. Chem. Soc., 70 (1948), 717.
5. Huppert, D., Kolodney, E., and Gutman, M., in Picosecond Phenomena, II, ed. C.V. Shank (Springer, Berlin, 1978).
6. Erdey-Gruz, T., and Lengyel, S., in Modern Aspects of Electrochemistry, XII, eds. J. Bockris and B.E. Conway (Plenum Press, New York, 1977, Ch.1).
7. Kaemer, W.P., and Diercksen, G.H.F., Chem. Phys. Letts., 5 (1970), 463.
8. Kebarle, P., Searles, S.K., Zolla, A., Scarbrough, J., and Arshadi, M., J. Am. Chem. Soc., 89 (1967), 6393.
9. Huppert, D., and Kolodney, E., Chem. Phys., 63 (1981), 401.
10. Stillinger, F.H., Adv. Theoret. Chem., 5 (1977).
11. Narten, A.H., and Levy, H.A., J. Phys. Chem., 62 (1975), 1545.
12. Wiesenfeld, J.M., and Ippen, E.P., Chem. Phys. Letts., 73 (1980), 47.
13. Lie, G.C., and Clementi, E., J. Chem Phys., 62 (1975), 2195.
14. Stillinger, F.H., and Rahman, A., ibid., 61 (1974), 1541.

15. Searcy, J.Q., and Fenn, J.B., ibid., 61 (1974), 5282.
16. Newton, M.D., and Ehrenson, S., J. Am. Chem. Soc., 93 (1971), 4971.
17. Kebarle, P., "Thermodynamic information from gas phase ion equilibria. Interaction between ions and molecules," Nato Adv. Study Institute Ser. B, Phys. ed. P. Auslous (Plenum Press, New York, 1975).
18. Schulman, S.G., and Vogt, B.S., J. Phys. Chem., 85 (1981), 2074.
19. Vogt, B.S., and Schulman, S.G., Chem Phys. Letts., 95 (1983), 151.
20. Vogt, B.S., and Schulman, S.G., ibid, 99 (1983), 157.
21. Huppert, D., Kolodney, E., Gutman, M., and Nachiel, E., J. Am. Chem. Soc., 104 (1982), 6249.
22. Eigen, M., Krase, W., Moas, G., and DeMayer, L., in Progress in Reaction Kinetics, II, ed. G. Porter (Pergamon Press, New York, 1964, Ch.6).
23. Conway, B.E., in Modern Aspects of Electrochemistry, III, eds. J. Bockris and B.E. Conway (Plenum Press, New York, 1977, Ch.3).
24. Gutman, M., Huppert, D., and Nachliel, E., Eur. J. Biochem., 121 (1982), 637.
25. Gutman, M., Nachliel, E., and Huppert, D., ibid., 121 (1982), 175.
26. Bardez, E., Goguillon, B., Keh, E., and Valeur, B., J. Phys. Chem., 88 (1984), 1909.
27. Kubik, R., Heike, H.F., and Jönsson, B., Helv. Chim. Acta, 65 (1982), 170.
28. Jönsson, B., and Wennerström, H., J. Colloid. Interface Sci., 89 (1982), 428.
29. Bardez, E., Monnier, E., and Valeur, B., J. Phys. Chem., 89 (1985), 5031.
30. Politi, M.J., and Chaimovich, H., J. Soln. Chem., 18 (1989), 1055.

31. Huppert, D.H., Jayaraman, R.G., Maines, R.G., Sr., Steyert, D.W., and Rentzepis, P.M., J. Chem. Phys., **81** (1984), 5596.
32. Lee, J., Griffin, R.D., and Robinson, G.W., *ibid.*, **82** (1985), 4920.
33. Kolthoff, Z.M., and Rosenblum, C., Acid-Base Indicators (Macmillon, New York, 1977).
34. Lee, J., and Robinson, G.W., J. Chem. Phys., **81** (1984), 1203.
35. Robinson, G.W., Lee, J., and Moore, R.A., in Picosecond Phenomena, IV, eds. D.H. Auston and K.B. Eisenthal (Springer, Berlin, 1984, p.313).
36. Lee, J., and Robinson, G.W., J. Am. Chem. Soc., **107** (1985), 6153.
37. Wiche, E., Eigen, M., and Ackermann, Th., Z. Phys. Chem. (N.F.), **1** (1954), 340.
38. Eigen, M., and DeMaeyer, L., Proc. R. Soc., A, **247** (1958), 505.
39. Eigen, M., in The Structure of Electrolyte Solutions, ed. W.J. Hamer (Wiley, New York, 1959).
40. Shizuka, H., Ogiwara, T., Narita, A., Sumitani, M., and Yoshihara, K., J. Phys. Chem., **90** (1986), 6708.
41. Maeda, M., and Ohtaki, H., Bull. Chem. Soc. Jpn., **48** (1975), 3755.
42. Narten, A.H., Vaslow, F., and Levy, H.A., J. Chem. Phys., **58** (1971), 5017.
43. Pines, E., Huppert, D., Gutman, M., Nachiel, N., and Fishman, M., J. Phys. Chem., **90** (1986), 6708.
44. Lim, E.C., in Excited States III, ed. E.C. Lim (Academia, New York, 1977, p. 326-328).
45. El-Sayed, M.A., and Kasha, M., Spectrochim. Acta, **15** (1959), 758.
46. Noe, L.J., Degen-Kolb, E.D., and Rentzepis, P.M., J. Chem. Phys., **68** (1978), 4835.

47. Robinson, G.W., Lee, J., Casey, K.G., and Statman, D., Chem. Phys. Letts., 123 (1986), 485.
48. Robinson, G.W., Thistlethwaite, P.J. and Lee, J., J. Phys. Chem., 90 (1986), 4224.
49. Lee, J., Robinson, G.W., Webb, S.P., Phillips, L.A., and Clarke, J.H., J. Am. Chem. Soc., 108 (1986), 6538.
50. Lee, J., Robinson, G.W., and Bassez, M.-P., ibid., 108 (1986), 7477.
51. Kamb, B., in Structural Chemistry and Molecular Biology, eds. A. Rich and N. Davidson (Freeman, San Francisco, 1968, p. 507).
52. Triolo, R., and Narten, A.H., J. Phys. Chem., 63 (1975), 3624.
53. Hasted, J.B., in Aqueous Dielectrics, (Chapman and Hall, London, 1973, Ch. 2 and 3).
54. Eisenburg, D., and Kauzman, W., in The Structure and Properties of Water, (Clarendon Press, New York, 1969, p. 224).
55. Kell, G.S., in Water, A Comprehensive Treatise, I, ed. F. Franks (Plenum Press, New York, 1972, Ch.10).
56. Lang, E., and Lüdeman, H.-D., J. Chem. Phys., 67 (1977), 718.
57. Lang, E., and Lüdeman, H.-D., Ber. Bunsen-Ges. Phys. Chem., 84 (1980), 462.
58. Lang, E., and Lüdeman, H.-D., ibid., 85 (1981), 603.
59. Angell, C.A., in Water, A Comprehensive Treatise, II, ed. F. Franks (Plenum Press, New York, 1982, Ch.1).
60. Angell, C.A., Oguni, M., and Sichina, W.J., J. Phys. Chem., 86 (1982), 998.
61. Oguni, M., and Angell, C.A., J. Chem. Phys., 78 (1983), 7334.
62. Eigen, M., and Kustin, K., J. Am. Chem. Soc., 82 (1960), 5952.

63. Harned, H.S., and Owen, B.B., The Physical Chemistry of Electrolyte Solutions, III (Reinhold, New York, 1958).
64. Webb, S.P., Philips, L.A., Yeh, S.W., Tolbert, L.M., and Clark, J.H., J. Phys. Chem., 90 (1986), 5154.
65. Eigen, M., Angew. Chem. Int. Ed. Engl., 3 (1964), 1.
66. Lau, Y.K., Ikuta, S., and Kebarle, P., J. Am. Chem. Soc., 104 (1982), 1462.
67. Del Bene, J.E., Mettel, H.D., Frisch, M.J., Luke, B.T., and Pople, J.A., J. Phys. Chem., 87 (1983), 3277.
68. Frisch, M.J., Pople, J.A., and Del Bene, J.E., ibid., 89 (1985), 3664.
69. Del Bene, J.E., Frisch, M.J., and Pople, J.A., ibid., 89 (1985), 3669.
70. Clementi, E., ibid., 89 (1985), 4426.
71. Lee, J., J. Am. Chem. Soc., 111 (1989), 427.
72. Swinehart, J.H., Rodgers, T.E., and Taube, H., J. Chem. Phys., 38 (1963), 398.
73. Krishnan, R., Fillingim, T.G., Lee, J., and Robinson, G.W., J. Am. Chem. Soc., 112 (1990), 1353.
74. Schulman, S.G., Kelly, R.N., and Gonzales, N.J., Pure and Appl. Chem., 59 (1987), 655.
75. Schulman, S.G., Kelly, R.N., and Chen, S., Chim. Oggi, 8 (1990), 13.
76. McClellan, A.L., Dipole Moments, (Freeman, San Francisco, 1963).
77. Bjerrum, N., Dan. Mat. Fys. Medd., 27 (1951), 1.
78. Bernal, J., and Fowler, R., J. Chem. Phys., 1 (1933), 515.
79. Pauling, L., The Nature of the Chemical Bond, 3rd ed. (Cornell University Press, New York, 1960).
80. Frank, H.S., and Wen, W.Y., Disc. Faraday Soc., 24 (1957), 133.

81. Danford, M.D., and Levy, H.A., J. Am. Chem. Soc., 84 (1982), 3965.
82. Franks, H.S., in Water, A Comprehensive Treatise, I, ed. F. Franks (Plenum Press, New York, 1972, Ch. 10).
83. Kell, G.S., in Water and Aqueous Solutions, Ed. R.A. Horne (Wiley-Interscience, New York, 1972, p. 331).
84. Davis, C.M., and Jarzynski, J., *ibid.*, p. 337.
85. Wang, J.H., J. Am. Chem. Soc., 73 (1951), 4181.
86. Wang, J.H., Robinson, C.V., and Edelman, I.S., *ibid.*, 75 (1953), 466.
87. Davis, C.M., and Jarzynski, J., Adv. Mol. Relax. Proc., 1 (1967-1968), 155.
88. Sarkisov, G.W., Malenkov, G.G., and Dashevsky, K., Mol. Phys., 27 (1974), 1249.
89. Owicki, J.C., and Scheraga, H.A., J. Phys. Chem., 82 (1978), 1257.
90. Stillinger, F.H., and Rahman, A., J. Chem. Phys., 63 (1975), 5233.
91. Stillinger, F.H., and Rahman, A., *ibid.*, 68 (1978), 666.
92. Reichardt, C., Solvents and Solvent Effects in Organic Chemistry, 2nd ed. (VCH, New York, 1988, p. 1-66).
93. Daudel, R., Quantum Theory of Chemical Reactivity, (Reidel Dordrecht, Boston, 1973).
94. Kortum, G., Lehrbuch der Electrochemie, 5th ed. (Verleg Chemie, Weinheim, 1972).
95. Friedman, H.L., and Krishnan, C.V., in Water, A Comprehensive Treatise, III, ed. F. Franks (Plenum Press, New York, 1973, Ch. 1).
96. Criss, C.M., and Solomon, M., Physical Chemistry of Organic Solvent Systems, ed. A.K. Covington and T. Dickinson (Plenum Press, New York, 1973).
97. Smith, D.W., J. Chem. Educ., 54 (1977), 540.

98. Marcus, Y. Rev. Anal. Chem., 53 (1980), 5.
99. Marcus, Y., *ibid.*, 58 (1986), 1721.
100. Eley, D.D., Trans. Faraday Soc., 35 (1939), 1281.
101. Frank, H.S., and Evans, M.W., J. Chem. Phys., 13 (1945), 507.
102. Némethy, G., and Scheraga, H.A., *ibid.*, 36 (1962), 3382.
103. Krishnan, C.V., and Friedman, H.L., J. Phys. Chem., 73 (1969), 1572.
104. Arnett, E.M., Kover, W.B., and Carter, J.V., J. Am. Chem Soc., 91 (1969), 4028.
105. Friedman, M.E., and Scheraga, H.A., J. Phys. Chem., 69 (1965), 3795.
106. Arnett, E.M., and McKelvey, D.R., in Solute-Solvent Interaction, eds. J.F. Coetzee and C.D. Ritchie (Dekker, New York, 1969).
107. Cennamo, F., and Tartaglione, E., Nuovo Cimento, 11 (1959), 401.
108. Bale, H.D., Sheplar, R.E., and Sorgen, D.K., Phys. Chem Liq., 1 (1968), 181.
109. Franks, F. and Reid, D.S., in Water, A Comprehensive Treatise, II, ed. F. Franks (Plenum Press, New York, 1973, Ch. 5).
110. Zachariasen, W.H., J. Chem. Phys., 3 (1935), 158.
111. Tucker, E.E., and Christian, S.D., J. Phys. Chem., 81 (1977), 1295.
112. Fletcher, A.N., and Heller, C.A., *ibid.*, 71 (1967), 3742.
113. Anderson, B.D., Rytting, J.H., and Higuchi, T., Int. J. Pharm., 1 (1978), 15.
114. Tucker, E.E., Farnham, S.B., and Chritian, S.D., J. Chem. Phys., 73 (1969), 3820.
115. Anderson, B.D., Rytting, J.H., Lindenbaum, S., and Higuchi, T., *ibid.*, 79 (1975), 2340.

116. Krestov, G.A., Thermodynamics of Solvation, ed. J. Burgess (Ellis Horwood, New York, 1991).
117. Mikhailov, V.A., J. Struct. Chem., 9 (1968), 332.
118. Mikhailov, V.A., and Ponomarova, L.I., *ibid.*, 9 (1968), 8.
119. Potts, A.D., and Davidson, D.W., J. Phys. Chem., 69 (1965), 996.
120. Kortum, G., and Weller, A., Z. Naturforsch., 5a (1950), 451.
121. Franks, F., and Ives, D.J.G., Quart. Rev., 20 (1966), 996.
122. Gurney, R.W., Ionic Processes in Solution, (Dover, New York, 1962).
123. Samoilov, O. Y., Structure of Aqueous Electrolyte Solutions and the Hydration of Ions, (Plenum Press, London, 1965).
124. Frohlich, H., Theory of Dielectrics, (Oxford University Press, Oxford, 1949).
125. Barbara, P.F., and Jarzeba, W., in Advances in Photochemistry, XV, eds. D.H. Volman, G.S. Hammond, and K. Gollnick (J. Wiley and Sons, Inc., New York, 1990, Ch.1).
126. Pottel, R., in Water, A Comprehensive Treatise, III, ed. F. Franks (Plenum Press, New York, 1973, Ch.3).
127. Onsager, L., Can. J. Chem., 55 (1977), 1819.
128. Hubbard, J., and Onsager, L., J. Chem. Phys., 67 (1977), 4850.
129. Calef, D.F., and Wolynes, P.G., *ibid.*, 78 (1983), 4145.
130. Kosower, E.M., and Huppert, D., Chem. Phys. Letts., 96 (1983), 433.
131. Castner, E.W., Jr., Maroncelli, M., and Fleming, G.R., J. Chem. Phys., 86 (1987), 1090.
132. Caldin, E.F., and Gold, V., Proton Transfer Reactions,

(Chapman and Hall, London, 1985).

133. Beckey, H.D., Z. Naturforsch., 14a (1959), 712.
134. Knewstubb, P.F., and Ticker, A.W., J. Chem. Phys., 38 (1963), 464.
135. Lundgren, J.O., and Williams, J.W., *ibid.*, 58 (1973), 788.
136. Conway, B.E., Bockris, J. O'M., and Linton, H., *ibid.*, 24 (1956), 834.
137. Wei, S., Shi, Z., and Castleman, A.W., Jr., *ibid.*, 94 (1991), 3268.
138. Erdey-Grúz, T., and Lengyel, S., in Modern Aspects of Electrochemistry, XII, eds. J.O'M. Bockris and B.E. Conway (Plenum Press, New York, 1977, Ch.1).
139. Forster, T., Z. Elektrochem., 54 (1950), 42.
140. Weber, K., Z. Phys. Chem., 815 (1931), 18.
141. Weller, A., Z. Elektrochem., 56 (1952), 662.
142. *Ibid.*, 61 (1957), 956.
143. Brønsted, J.N., Z. Phys. Chem., 102 (1922), 169.
144. *Ibid.*, 115 (1925), 337.
145. Lakowicz, J.R., Principles of Fluorescence Spectroscopy, (Plenum, New York, 1983, Ch.3).
146. Rentzepis, P.M., Annu. Rev. Phys. Chem., 24 (1973), 473.
147. O'Connor, D.V., and Phillips, D., Time-Correlated Single Photon Counting, (Academic, Orlando, 1984).
148. Laws, J.R., and Brand, L., J. Phys. Chem., 83 (1979), 795.
149. Birks, J.B., Photophysics of Aromatic Molecules, (John Wiley and Sons, New York, 1970).
150. Loken, M.R., Hayes, J.W., Gohlke, J., and Brand, L., Biochem., 11 (1972), 4779.

151. Kelly, R., Doctoral Dissertation, University of Florida (1986).
152. Schulman, S.G., Threatte, R.M., Capomacchia, A.C., and Paul, W.L., J. Pharm. Sci., 63 (1974), 876.
153. Pant, D., Tripathi, H.B., and Pant, D.D., J. Photochem. Photobiol., A: Chem., 54 (1990), 239.
154. Suzuki, H., Electronic Absorption Spectra and Geometry of Organic Molecules, (Academic Press, New York, 1967, pp. 262-284).
155. Imamura, A., and Hoffman, R., J. Am. Chem. Soc., 90 (1968), 5379.
156. Bridges, J.W., Creaven, P.J., and Williams, R.T., J. Biochem., 96 (1965), 872.
157. Adrien, A., and Sergeant, E.P., The Determination of Ionization Constants, 3rd ed., (Chapman and Hall, London, 1984; p. 145).
158. Badea, M.G., and Brand, L., Methods Enzymol., 61 (1971), 378.
159. Yguerabide, J., *ibid.*, 62 (1972), 498.
160. Eaton, D.F., J. Appl. Chem., 62 (1990), 1631.
161. Harris, C.M., and Selinger, B.K., J. Phys. Chem., 84 (1980), 891.
162. Lakowicz, J.R., Principles of Fluorescence Spectroscopy, (Plenum, New York, 1983, Ch.9).
163. Davis, G.A., J.C.S. Chem. Commun., (1973), 728.
164. Ireland, J.F., and Wyatt, P.A.H., Adv. Phys. Org. Chem., 12 (1976), 131.
165. Im, H-S., and Bernstein, E.R., J. Phys. Chem., 88 (1988), 7337.
166. Dick, B., and Hohlneicher, G., Phys. Chem., 94 (1985), 131.
167. Swiderek, P., Michaud, H., Hohlneicher, G., and Sanche, L., Chem. Phys. Letts., 187 (1991), 583.

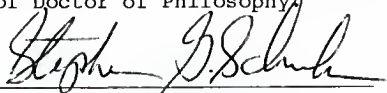
168. Klevens, H.B., and Platt, J.R., J. Chem. Phys., 17 (1949), 470.
169. Schulman, S.G., Vincent, W.R., and Underberg, W.J.M., J. Phys. Chem., 85 (1981), 4068.
170. Allan, M., J. Elect. Spec., 48 (1989), 219.
171. Von Smoluchowski, M., Z. Phys. Chem., 92 (1917), 129.
172. Naberukhin, Y.I., and Rogov, V.A., Russ. Chem. Rev., 40 (1971), 207.
173. Hynes, J.T., J. Stat. Phys., 42 (1986), 149.
174. Kosower, E., and Huppert, D., Ann. Rev. Phys. Chem., 37 (1986), 127.
175. Simon, J.D., Acc. Chem. Res., 21 (1988), 128.
176. Knochenmuss, R., Leutwyler, S., J. Chem. Phys., 91 (1989), 1268.
177. Frank, I.M., and Vavilov, S.I., Z. Phys., 69 (1931), 100.
178. Schulman, S.G., Townsend, R., Kimura, S., and Chen, S., Anal. Chim. Acta, 227 (1989), 165.
179. Butler, J.A.V., Thomson, D.W., and MacLennan, W.H., J. Chem. Soc., (1933), 674.
180. Christian, S.D., Lane, E.H., and Tucker, E.E., J. Soln. Chem., 10 (1981), 181.
181. Nord, L., Tucker, E.E., and Christian, S.D., *ibid.*, 13 (1984), 849.
182. Okamoto, B.Y., Wood, R.H., and Thompson, P.J., J. Chem. Soc. Faraday Trans. I, 74 (1978), 1990.
183. Goates, J.R., and Sullivan, R.J., J. Phys. Chem., 62 (1958), 188.
184. Lam, S.Y., and Benoit, R.L., Can. J. Chem., 52 (1974), 718.
185. Kangro, W., and Groenveld, A., Z. Phys. Chem., 33 (1961), 11.

186. French, H.T., J. Chem. Thermodyn., 19 (1987), 1155.
187. Debye, P., and Hückel, E., Physik Z., 24 (1923), 185.
188. Akerlof, G., J. Am. Chem. Soc., 54 (1932), 4123.
189. MacInnes, D.A., The Principles of Electrochemistry; (Reinhold, New York, 1939, Ch.7).
190. Robinson, R.A., and Stokes, R.H., Electrolyte Solutions, 2nd Ed.; (Academic Press, New York, 1959, Ch.4).

BIOGRAPHICAL SKETCH

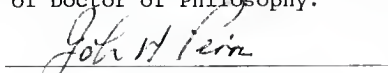
Robert W. Townsend was born on November 25, 1953, to Leland H. and Frances L. Townsend. He graduated from Lynn Classical High School in 1971. He graduated from Florida Junior College in 1977 with an Associate of Science degree in nursing and has practiced as a registered professional nurse since. In 1984, he graduated from the University of North Florida with a Bachelor of Arts degree in chemistry. The following year he entered graduate school at the University of Florida in the College of Pharmacy. He received his Doctor of Philosophy degree in pharmaceuticals in 1992 under the guidance of Dr. S. G. Schulman.

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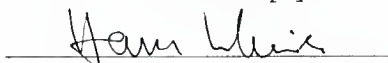
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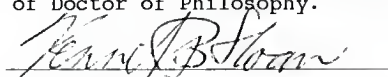
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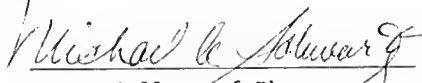
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Kenneth Sloan
Associate Professor
of Medicinal Chemistry

This dissertation was submitted to the Graduate Faculty of the College of Pharmacy and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Dean, College of Pharmacy

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